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A comparison of the diet and health of pre-menopausal Indian and Caucasian vegetarian women.

Reddy, Sheela

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**A COMPARISON OF THE DIET AND HEALTH
OF PRE-MENOPAUSAL INDIAN AND CAUCASIAN
VEGETARIAN WOMEN**

**by
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**A thesis submitted to the University of London
for the Degree of Doctor of Philosophy
in the Faculty of Science**

**August 1991
Department of Nutrition and Dietetics
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**Dedicated to the memories of
my Father
and Suresh**

ABSTRACT

The diet and health of the Indian ethnic minority in the UK is reviewed and a study is presented in which the health and diet of pre-menopausal Indian women is compared with that of Caucasian vegetarians and omnivores.

The diet of the Caucasian vegetarians was generally adequate with regard to most nutrients with the exception of vitamin B₁₂. The Indian vegetarian diets were low in vitamin B₁₂, folate, copper, zinc and α -linolenic acid. The proportion of energy derived from fat and saturated fatty acids was greater in the omnivores and energy from polyunsaturated fatty acids was greater in the vegetarians. The fatty acid intakes were confirmed by the analyses of total plasma lipids and the phospholipid and free fatty acid fractions. The dietary fibre intakes were the greatest in the Caucasian vegetarians and similar in the Indians and the Caucasian omnivores.

The Indians had higher BMI and skinfold thickness, despite their lower energy intakes. Haemoglobin concentrations were inside the normal range in all the groups. Vitamin B₁₂ and ferritin concentrations were lower in the Indians. It is concluded that both the vegetarian groups are at risk of nutritional anaemias and need to ensure adequate intakes of vitamin B₁₂ and iron.

The Indian vegetarians had significantly lower levels of retinol in plasma compared to the Caucasians. There was a positive correlation between the intakes of α -tocopherol and its concentrations in plasma, which were significantly lower in the Indians and the omnivores compared to the Caucasian vegetarians.

Plasma concentrations of total cholesterol, LDL-cholesterol and Apo A1 were significantly greater in the omnivores compared to the Caucasian vegetarians but not the Indian vegetarians. The Indians differed from both groups of Caucasians by their lower levels of HDL and HDL₂-cholesterol and ^{higher levels of} apo(a) which is a constituent of Lp(a). Significant relationships between centripetal distribution of body fat and plasma lipoproteins, and dietary intake of saturated fatty acids and plasma cholesterol were found.

No differences in the total and free oestradiol concentrations in plasma were found between the groups but the Caucasian vegetarians had significantly greater levels of both total and free testosterone. Sex hormone binding globulin was lower in the Indians and was related to centripetal body fat.

The faecal concentrations of secondary bile acids were lower in the Indians but the total output did not differ between groups. There was a decreased degradation of faecal primary bile acids and neutral sterols in the Indians compared to the Caucasians despite their lower fibre intake, which was related to the lower faecal pH and sulphur/nitrogen ratio. These results were consistent with the lower risk of colon cancer in the Indians.

The adjusted mean birth weight of infants born to Indian vegetarians was lower compared to the Caucasians and the difference could not be explained by the effects of maternal smoking or stature. Analysis of cord plasma and arteries revealed lower proportions of DHA (22:6n-3) and higher proportions of 22:5n-6 in the Indians, but there was no relationship between these variables and birth weight.

It is concluded that the general health of Indian vegetarians is satisfactory, but there is a need to target health and nutritional information at this group, taking into account their cultural and religious practices.

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- Reddy, S. and Sanders, T.A.B. (1990). Haematological studies on pre-menopausal Indian and Caucasian vegetarians compared with Caucasian omnivores. *British Journal of Nutrition* **64**: 331-338.

LIST OF ABBREVIATIONS

25(OHD)	25-Hydroxycholecalciferol
Apo A1 & A2	Apolipoprotein A1 and A2
Apo(a)	Apolipoprotein(a)
ApoB	Apolipoprotein B
ApoC	Apolipoprotein C
ApoE	Apolipoprotein E
ATP	Adenosine Triphosphate
BMI	Body Mass Index
CCK	Cholecystokinin
CHD	Coronary Heart Disease
CI	Confidence Interval
CO	Caucasian Omnivores
CV	Caucasian Vegetarians
DHA	Docosahexaenoic Acid (22:6n-3)
DNA	Deoxyribonucleic Acid
E₂	Oestradiol
ECG	Electrocardiogram
EDTA	Ethylenediamine Tetraacetic Acid
EFA	Essential Fatty Acid
EPA	Eicosapentaenoic Acid (20:5n-3)
FAO	Food and Agricultural Organisation
FFA	Free Fatty Acids
GLC	Gas-Liquid Chromatography
Hb	Haemoglobin
HDL	High Density Lipoprotein
HPLC	High Performance Liquid Chromatography

List of Abbreviations

continued

IARC	International Association for Research on Cancer
ICRF	Imperial Cancer Research Fund
IDDM	Insulin Dependent Diabetes Mellitus
IDL	Intermediate Density Lipoprotein
IV	Indian Vegetarians
LCAT	Lecithin : Cholesterol Acyltransferase
LDL	Low Density Lipoprotein
LFS	Labour Force Survey
Lp(a)	Lipoprotein(a)
LRC	Lipid Research Clinic
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
MCV	Mean Cell Volume
MUFA	Monounsaturated Fatty Acids
NIDDM	Non-Insulin Dependent Diabetes Mellitus
NRC	National Research Council (USA)
NSP	Non-Starch Polysaccharides
P:S	Polyunsaturated : Saturated Fatty Acids Ratio
PCV	Packed Cell Volume
PFA	Phospholipid Fatty Acid
Pg	Progesterone
PMR	Proportional Mortality Ratio
PUFA	Polyunsaturated Fatty Acids
RDW	Red Cell Distribution Width
RNI	Reference Nutrient Intake

List of Abbreviations

continued

S/N	Sulphur/Nitrogen Ratio
SCFA	Short Chain Fatty Acids
SDA	Seventh-Day Adventists
SEM	Standard Error of the Mean
SFA	Saturated Fatty Acids
SHBG	Sex Hormone Binding Globulin
SMR	Standardised Mortality Ratio
T	Testosterone
TEFA	Total Esterified Fatty Acid
VLDL	Very Low Density Lipoprotein
W/H Ratio	Waist/Hip Ratio
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

PART I

INTRODUCTION

The United Kingdom today is a multiracial, polyethnic society integrated under a state system (Carlson *et al.*, 1984). Substantial numbers of migrants of varied ethnic origins have settled in Europe over the past thirty years mainly from the former colonies. Apart from the Irish, who in 1981 constituted the largest single group of immigrants to the U K, most of the immigrants are either from the Indian subcontinent, the West Indies or Africa (OPCS, 1981).

1:1 The Asian Community

The term "Asian" is generally used in the U.K. to describe people originating from India, Pakistan, Bangladesh or Sri Lanka, while in the U.S.A. people from South-East Asia or Japan are referred to as Asians. However, the term is too inclusive and unsatisfactory. A common Asian identity is formed by circumstances rather than any inherent similarities (Karseras & Hopkins, 1987) and it is necessary to identify the different groups which comprise the Asian community originating from the Indian subcontinent.

Pakistanis

The Islamic state of Pakistan was formed in 1947 with the end of British colonial rule of the Indian subcontinent. A majority of Pakistanis migrated to Britain from post-independence turbulence to escape economic uncertainty caused by the partition of India. Most Pakistanis are followers of Islam (Muslims), a monotheistic religion founded by the prophet Mohammed in the seventh century. Islam is central to lifestyle and governs every activity undertaken by its followers.

Islamic behaviour is learnt from the holy book "*The Koran*", which states the Islamic food laws. It decrees that only flesh of cloven footed and cud chewing animals should be eaten and forbids the consumption of pork and blood of any animal. All animals have to be killed by ritual slaughter by slitting the jugular vein and draining the blood (Halal meat). Fish with scales and fins are allowed, however there is a prohibition on eating shellfish. Alcohol is also strictly prohibited.

Another edict that a devout Muslim should follow is fasting during the ninth month of the lunar year, known as Ramadan, during which the Koran is thought to have been written. Boys are expected to start fasting at 15 years and girls at 12 years of age. The fast demands that no food or drink should be taken between dawn and sunset. Pregnant and lactating women, incurably sick and the elderly are exempted from the fast. Fasting is thought to increase a Muslim's realisation of other people's difficulties and bring him closer to God and the more orthodox tend to conform regardless. This may have an impact on the health and nutritional status of the vulnerable groups among the Muslims. Muslim women generally do not enjoy as much freedom as other Asian women and are likely to be confined within their homes, few seeking outside employment.

Bangladeshis

Bangladeshis are also Muslims and originate from Bangladesh, the north-eastern part of the subcontinent, formerly known as East Pakistan until independence in 1971. Bangladeshi families are the most recent settlers in Britain and are still in the early stages of adjustment. Dietary laws are similar to those observed by Pakistanis, but as Bangladesh lies on the Ganges delta fish is an important source of nutrients. In accordance with Islamic law only fish with fins and scales are eaten. Few of the women are able to speak English and are therefore likely to be more isolated.

Indians - Gujaratis

The Gujarati community originates from the state of Gujarat on the Northwest coast of India. A substantial number of Gujaratis are from East Africa, where they had first migrated to the then British colonies. Approximately 10% are Muslims but a majority are Hindus. The Hindu religion has its origin in pre-vedic times (1500 BC). Hinduism is largely practised in the home and has no set of doctrines. It could be described as "a coalition of many teachings and practices" (Bowker 1983), but however a measure of uniformity is kept in the religion by reference to the Sanskrit texts (Vedas, Manusmriti, etc.). These clarify the role of social orders or castes (Brahmins - priests, Kshatriyas - warriors and administrators, Vaishnavas - traders and Sudras - those who undertake menial tasks in society). The majority of Gujaratis come from middle ranking castes and there are further divisions into sub-castes based on occupation. Most Gujaratis still practise caste endogamy. Hinduism deviates from other religions in its caste system and the concept of reincarnation. A Hindu ultimately hopes to achieve unity with his

creator but must go through many cycles of birth, death and reincarnation, each life being determined by the deeds of his previous existence. Prayer is an important aspect of Hinduism and it may be conducted at home or at the Temple. There are many sects and sub-sects, each with its own place of worship.

Dietary restrictions stem from a reverence for life. Orthodox Hindus believe in the doctrine of "Ahimsa" (not killing) and this, together with the sanctity of the cow, forms the basis of their vegetarian food habits. Many are vegetarian but milk and its products are eaten because they do not involve taking the animal's life, indeed they are highly prized, particularly ghee (clarified butter), which is believed to sanctify food cooked with it. Some do eat meat, but never beef as the cow is sacred and pork because like muslims is considered to be unclean. If fish is eaten the preference is for fish with scales and white fish. Alcohol is not forbidden but is regarded as a pollutant because it challenges the value of self-control. The more rigidly a Hindu adheres to a vegetarian diet, the more likely he is to better himself.

Abstention from eating food is a much praised virtue among Hindus. Fasting is included in religious observances and some spend two or three days a week fasting. Sometimes total abstinence from food is required during a 'fast', but some 'fasts' entail taking only pure foods such as milk, fruit, nuts and starchy root vegetables. Vows of abstention from certain foods are believed to influence events. Hinduism generally places few constraints on integration, especially with regard to women who expect to work outside the home and will probably continue to do so after the arrival of children. Employment aspirations are similar to the natives, although owning a business is seen as highly desirable (Karseras & Hopkins, 1987).

Indian Punjabis - Sikhs

The majority of Sikhs come from the Indian state of Punjab. Sikhism is an offshoot of the Hindu religion founded by Guru Nanak in the late 15th century. It combines concepts from both Hinduism and Islam. It is a monotheistic religion like Islam and unlike Hinduism has no caste divisions, but the belief of reincarnation is common to both. Uncut hair is one of the five signs of Sikhism - the others being, a comb worn under the turban, a sacred undergarment, a small dagger and most importantly a metal bangle which must always be worn. Although Sikhs repudiate the caste system

there are social divisions, such as agrarian farmers and craftsmen, and the groups generally do not intermarry.

Sikhs observe few dietary prohibitions, although those who have made an extra commitment to their religion promise to abstain from meat, fish, eggs, alcohol and tobacco. Most Sikhs avoid eating both beef and pork although there is no clear prohibition. They should ideally eat 'Jhatka' meat, which is from an animal killed at one stroke by decapitation. Fasting follows a similar pattern to Hindus but each has its own day set aside for this purpose. Sunday is kept as a holy day when Sikhs go to the temple (Gurudwara) for corporate worship. A meal prepared by the women is an integral part of Sunday at the Gurudwara. Generally Sikhs take a liberal view in the matters of eating and are easily adaptable to their surroundings. Women in Sikh homes have considerable freedom and are able to worship with men and are given the same education. Many Sikh women pursue employment outside.

1:2 History of Migration

Migration from the sub-continent to the U.K. began during the second world war, most being sailors who deserted their ships and moved inland to work in the Midlands factories. The subsequent arrival of migrants from the sub-continent was in response to demand for labour during the rebuilding of post-war Britain. Initially most were economically active single men who readily found employment in the manufacturing industries particularly the foundries, textile mills, transport and health services. A gradual build-up of immigration was observed during the next decade when the original migrants financed travel for other male relatives and villagers. This pattern of immigration has resulted in Indians originating from only a few areas within the sub-continent: Pakistan, Bangladesh and the Indian states of Punjab and Gujarat, settling in distinct communities, e.g. Sikhs in Southall (West London), Gujaratis in Leicester and Wembley (North London). The Commonwealth Immigration Act 1962 curtailed the flow of male migrants from India and Pakistan but those already here began to bring their dependents. Deakin (1970) noted that by 1967 over 90% of all commonwealth immigrants to Britain were dependents. In the 1970's Indian migration was composed mainly of East African Asians who came as political refugees from racist policies of their new rulers especially from Uganda in 1972. Indians from East Africa were often of higher social class than those who arrived directly from India. They had been lawyers,

teachers, administrators and merchants and soon settled down resuming their former occupations.

1:3 Population

Estimates of numbers of migrants are unsatisfactory. Despite a government white paper published in 1978, recognising the need for a question of ethnic origin in the 1981 census, the question was excluded as it was thought to be too sensitive. However, a question on the country of birth was included which gave estimates inclusive of the ethnically white former colonists and failed to identify ethnic minorities born in Britain. The Labour Force Survey conducted by the Office of Population Census and Surveys is currently the most important source of information on ethnic origin where respondents are asked to which ethnic group named in the list (White, West Indian or Guyanese, Indian, Pakistani, Bangladeshi, Chinese, African, Arab, Mixed Origin or other) they consider they belong. Using information from the 1986, 1987 and 1988 Labour Force Surveys, the total size of all ethnic minority population is estimated to be 2.58 million representing 4.7% of the total population of Great Britain compared with 3.9% in 1981 and 2.3% in 1971 (Haskey, 1990). Two-thirds of the increase is due to natural growth and one third to net immigration (Shaw, 1988).

People from the Indian sub-continent are the largest subgroup of non-white population numbering 1.26 million (51%) and Indians originating from Gujarat and Punjab are the largest single ethnic minority group (745,000; i.e. 30% of the total ethnic minorities). A high proportion (70%) of Indians are resident in the metropolitan areas and a majority in London and the Southeast. The London Borough of Ealing, which includes Southall, is the most populated (21,195) followed by Brent (13,923; 1981 Census, OPCS).

Almost without exception, the age profile of each ethnic minority population is younger than the white population for both males and females (Haskey, 1988). The sub-ethnic groups of Pakistanis and Bangladeshis are the youngest with around half of the male and female population being aged under 16, whilst the age distribution of Indians is more similar to that of the total population.

Sex composition of all groups of 'Asian' population can be attributed to a mixture of labour demands and responses to legislation. The proportion of women settlers was low, as shown in a study of Sikhs in Southall in the late 1950s which estimated that the proportion of women was as low as 4% (Aurora, 1967), but this deficiency later rebalanced in the 1970s and 1980s (Webster and Fox, 1989). In most ethnic minority populations males outnumber females, particularly in the Bangladeshi community with 131 males to every 100 females compared to 95 males in the general U.K. population. Indian and Pakistani communities are estimated to have 101 and 109 males to every 100 females respectively (Haskey, 1988). A majority of the female population with origins in the Indian sub-continent are of child bearing age, approximately 47% of the females being in the age groups of 16-29 and 30-44 years.

1:4 Socio-Economic Climate

Asian migration to the U.K. has been primarily for economic reasons to enhance their socio-economic status. Although this may have been possible in the 1950s - 1970s, the present economic climate in the U.K. has meant that Asians face numerous problems compounded by other factors such as difficulty in communication, discrimination, lack of qualifications etc.

Social Class

Social class as currently defined does not accurately reflect the status of Asians (Marmot *et al.*, 1984). This is probably because it does not take into account their original status, or their overall living standards. However, the data available shows that Asians are disproportionately distributed in the lower social classes III(b), IV and V.

Education and Employment

Asians have a high regard for education with comparatively larger proportions of full-time students than the general population (Central Statistical Office, 1986). Several studies have shown Asian pupils performing better or equal to whites (Carr-Hill and Chadha-Boreham, 1988). However, they are more likely to face unemployment, be over qualified for their jobs and receive lower wages for their skills (Labour Force Survey, 1987). Labour Force Surveys (LFS) have consistently shown that unemployment rates for white men and women are lower than the corresponding rates in ethnic minority groups. According to the LFS (1985), the unemployment rate for Indian men was 18%

compared to 11% in the white population while 28% of Pakistani/Bangladeshi men were unemployed. A similar pattern exists for women, with 15% of Indian women and 44% of Pakistani/Bangladeshi women being unemployed compared to only 10% white women. But these unemployment rates are based on only those who are registered as unemployed. The lack of employment opportunities, particularly for Asian women has led to an increase in piece-rate earnings, working at extremely low rates of pay, long hours and lack of benefits such as sickness and maternity benefits (Jordan & Waine, 1986).

Housing

Home ownership is much coveted by the Asians and according to Davies (1984), owner occupation is higher among Asians due to the 'risk taking element' or the 'enterprise spirit'. But it may be the outcome of immigrants reacting to a variety of restraints, economic and other, such as discrimination, language etc. Within these constraints, the rational response seems to be to maximise the use of personal and family resources, limit vulnerability and exposure to uncertainty and racism as well as to enhance their status (Luthera, 1988). Although 77% of the Asian population are owner-occupiers compared to 58% of the general population (OPCS, 1981), their houses are usually older, of a poorer quality and situated in over crowded areas which lack amenities (Smith, 1980). Council tenancy is the lowest in the Asian population at 18% compared to 31% of the general population. However, 31% of Bangladeshis were in council housing compared to only 13% Indians and 11% Pakistanis (OPCS, 1981).

1:5 The Asian Family and Lifestyle

"Asian families are seen to be patriarchal in structure, with a dominating male presence, a submissive mother, usually living in overcrowded housing and with inter-generational conflicts". Such assumptions are imbued with the conception of the 'ideal' family type of two (married) adults and one or two children, and there is a marked tendency to see other family patterns as deviant, even pathological, without considering the reasons for the patterns or the possibility that they, too, may have advantages (Donovan, 1977).

The traditional family system in the Indian sub-continent is the joint/extended family, unlike in the UK where the norm is for nuclear families. Because of migration and immigration restrictions, Asian extended families in the UK are less common than

in the countries of origin (Anwar, 1981). Asian households usually include two or three generations consisting of members of all age-groups. Some households in UK include adult unmarried siblings of the husband or in some cases married brothers living in the same house as a joint family.

The popular reason for continued preference of the extended family system is that it is both traditional and pragmatic, where members can depend on each other in time of need. Large households are relatively frequent amongst Indian and Pakistani/Bangladeshi households - one in five and two in five respectively contain six or more people compared to one in fifteen in other groups (Haskey, 1989). Hence, the average household size is also estimated to be greater than that of the general population which is 2.6 persons per household. Pakistanis/Bangladeshis have a greater average household size of 4.9 compared to 4 in the Indians.

In contrast with the UK society, which emphasises individual development, family responsibilities and duties are considered more important than individual wishes in an Asian family (Commission for Racial Equality, 1985). Asian customs and life-styles are closely linked with religion and adherence to these often influences their standing within their own society and may sometimes effect wider integration. Major adaptations have occurred as the degree of exposure to the host country has increased. There is, however, a strong desire to maintain a distinct cultural identity, rather than to assimilate and this is evident even among UK born Asians (Alibhai, 1989).

1:6 The Asian Diet

The vastness of the Indian sub-continent with its regional variation in climate and culture is reflected in diverse dietary practices. Similar diversity is evident in the UK among the ethnic groups of Indian origin. Food habits are an indicator of identity as much as dress, language or religion. Whenever migrants settle in a new habitat their food habits inevitably change some resulting from unavailability of certain foods or their high cost. Other changes may particularly occur in migrants from developing countries as a result of a variety of new unfamiliar foods. Hence, food habits are a part of migrants adjustment to a new environment. Food behaviour is essentially symbolic; it reflects not only religious beliefs but also many other facets of life from the division of

labour in the family through work, worship and play to the aspirations which the migrants entertain in their new homeland (Kalka, 1988).

Religion is undoubtedly the most potent influence on food choice. A quarter of the Asian population is vegetarian due to them being Hindus, while a considerable proportion of them avoid pork due to religious restrictions. In addition to the dietary guidelines laid by religion, cultural or non-religious food traditions also exist. Food is classified into 'hot' or 'cold' categories and is believed to have medicinal properties. Pregnant women are advised to avoid foods like paw-paw, chicken, green mango, etc. as they are considered to be 'hot' foods and may cause miscarriage. Rice, green gram, spinach, etc. are believed to have a cooling effect and are given during fever (Hunt, 1976).

The earlier immigrants did not have a full choice of traditional foods but with the arrival of Asians from Africa there is an increased demand for ethnic foods thus creating a need to import traditional foodstuffs. Shops selling Asian groceries are well supplied and are usually accessible. Muslim communities often contain butchers selling 'halal' meat from established abattoirs where animals are killed according to the religious dictum. However, imported items are relatively expensive and families may often spend a disproportionate amount of income on food.

Generally Asian diets are cereal based but the staple food consumed usually depends on the area from which they originate. Wheat is predominantly grown in the northern part of the sub- continent, therefore Punjabis and Sikhs consume more wheat. Bangladeshis are known to prefer rice as it is the main crop cultivated in the Ganges delta which forms a major part of Bangladesh. Gujaratis seem to consume both rice, wheat and also millet which are all cultivated in the state (Wharton *et al.*, 1984).

Traditional meal patterns may be maintained or modified. Asians usually consume large main meals but snacks, both traditional and western, are popular (Wharton *et al.*, 1983). Breakfast has become the most westernised meal, cereals often replacing more traditional foodstuffs. Only women and children are at home for the midday meal, which comprises leftovers from the previous day. The traditional family meal being eaten in the evening. Problems of food acceptability occur when eating outside the home,

especially in school or work canteens and hospitals; it is not unusual to see Asian workers relying on home-packed lunches.

Food Consumption

Information concerning the amount of specific foods consumed by the Asian population is limited. However a study of their food habits can give qualitative information concerning food consumption. Bull & Barber (1984) identified thirty-seven Asian vegetarian households from the National Food Survey and estimated individual food intake within the household (Table 1:1). This study indicated that Asian vegetarians consumed more flour, rice, butter and other fats, fruits and vegetables. Dairy foods are popular among the Asians when compared to other groups.

TABLE 1:1

Household food consumption in vegetarian household compared with the national average, 1979-1982 (oz per head/week)

	Non-Asian Vegetarians	Asian Vegetarians	All Households
<i>Milk and Milk Products (pints equiv.)</i>	4.1	5.9	4.5
<i>Cheese</i>	8.7	2.7	3.9
<i>Eggs (no.)</i>	3.1	0.9	3.7
<i>Butter</i>	3.1	11.4	3.8
<i>Margarine</i>	4.8	1.2	4.0
<i>Other Fats</i>	2.8	18.2	3.3
<i>Sugar and Preserves</i>	8.7	6.5	13.1
<i>Vegetables and Vegetable Products</i>	84.3	91.4	85.8
<i>Fruit and Fruit Products</i>	49.8	49.1	27.2
<i>Bread</i>	23.1	10.6	31.2
<i>Flour, Rice & Other Cereal Products</i>	36.3	83.1	24.3
<i>Beverages (tea, coffee, etc.)</i>	2.3	1.2	3.0
<i>Miscellaneous (soups, spreads, pickles)</i>	6.9	4.1	9.4
<i>Meat and Meat Products</i>	0	0	39.6
<i>Fish and Fish Products</i>	0	0	4.8

Source: Bull & Barber (1984).

Nutrient Intakes:

Although they are not indicated by ethnic origin in the National Food Survey, Asians were identified as those having Asian meals and/or purchasing Asian foods above the national average (Wenlock and Buss, 1977). However, due to bulk purchasing habits of the Asians and variation in family size within the sample it was not possible to estimate a realistic nutrient intake in this group. Nutrient intakes of Asians have been mostly reported in men and pregnant women.

Indian men (Gujaratis) have lower energy intake compared to the general population (Table 1:2). Although the proportion of energy from total fat is similar to Caucasians, the P:S ratio is higher. Dietary fibre intakes are marginally above that of the Caucasians.

TABLE 1:2

Mean nutrient intakes of Gujarati men compared to Caucasian controls and British men

Nutrient Intake per day	Gujarati* Men (n=20)	Caucasian* Men (n=20)	British Men** Nutritional Survey
<i>Energy (kcal)</i>	2222	2677	2450
<i>% Energy from Protein</i>	12.4	14.0	15.2
<i>% Energy from Carbohydrate</i>	49.5	46.2	41.6
<i>% Energy from Fat</i>	38.7	38.7	37.6
<i>% Energy from Alcohol</i>	2.2	3.5	6.9
<i>P : S Ratio</i>	0.46	0.26	0.4
<i>Dietary Fibre (g)</i>	27	23	24.9
<i>Calcium (mg)</i>	936	1109	940
<i>Iron (mg)</i>	15.4	15.1	13.7
<i>Vitamin B₁₂ (µg)</i>	1.7	6.4	7.3

Sources: * Miller *et al.* (1988)
 ** Gregory *et al.* (1990)

Nutrient intakes during pregnancy (Table 1:3) show that the energy intakes were lower in Muslim women but energy derived from protein was higher than in the other groups. Sikh women had higher intakes of calcium, retinol and dietary fibre. Daily intakes of all the nutrients, with the exception of dietary fibre in Sikh women and carbohydrates in all groups, were below the dietary intakes reported in non-pregnant British women. However, the data on sub-ethnic groups of Asian women was obtained by 24-hour recall, which is liable to be inaccurate as this method relies on the individual's memory and judgement of quantities of foods eaten.

1:7 General Health

1:7:1 Vital Statistics

Births to mothers born outside the U.K. fell from 12.3% of all live births in Great Britain in 1981 to 11.1% in 1988. A higher fertility rate is seen in the Asians possibly due to the younger age distribution of the Asian population. Overall fertility rates dropped between 1971 and 1981 in England and Wales and have since remained stable. However, rates for women born outside UK have in general continued to fall since 1981. The total period fertility rate for women born in Pakistan and Bangladesh, although still much higher than for women born in other countries, fell by 44% between 1971 and 1988 and the rates for women born in India and East Africa fell by 32% and 17% respectively (Central Statistical Office, 1990). This may be due to adaptation to U.K. lifestyle and the increased use of contraceptives. It is important to note that birthplace does not necessarily equate with ethnic group. In particular there are an increasing number of women from ethnic minority population in the younger child bearing ages who were themselves born in the U.K.

1:7:2 Mortality: The Major Indicator of Health

An analysis of mortality of immigrants in England and Wales followed the introduction in 1969 of a statement of place of birth on death certificates. The standardised mortality ratios (SMR) for those born in the New Commonwealth and Pakistan for all causes and two major cause groups: circulatory diseases which include coronary heart disease; and neoplasms (cancers) are shown in Figure 1:1. The most strikingly high SMRs for all causes are for males and females born in the African countries and for females born in the Indian sub-continent and the Caribbean for all causes and circulatory diseases. However, this analysis is based on country of birth and

TABLE 1:3

Mean daily nutrient intake during pregnancy by ethnic sub-groups of Indian women compared to non-pregnant British women

Nutrients	Pakistani* Muslims (n=90)	Sikhs* (n=36)	Hindus* (n=29)	Bangladeshi* Muslims (n=10)	British** non-pregnant Women
Energy (kcal)	1589	1800	1790	1555	1680
Protein (g)	56	53	60	59	62
% Energy from Protein	14.1	11.8	13.4	15.2	15.2
Fat (g)	59	60	72	52.0	73.5
% Energy from Fat	33.4	30.0	36.2	30.1	39.2
Carbohydrate (g)	221	253	240	227	193
% Energy from Carbohydrate	52.2	52.7	50.3	54.7	43
Dietary Fibre (g)	17	21	16	12	18.6
Calcium (mg)	791	912	812	496	726
Iron (mg)	11	12	10	9	10.5
Copper (mg)	1.1	1.2	1.1	0.7	1.23
Zinc (mg)	7.3	6.4	7.2	6.7	8.4
Retinol (µg)	494	753	680	322	1413
Vitamin D (µg)	0.9	0.98	1.3	1.51	2.51
Thiamin (mg)	1.07	1.21	0.97	0.51	1.24
Riboflavin (mg)	1.15	1.39	1.28	0.91	1.57
Niacin (NE)	21.5	22.0	22.0	18.2	28.5
Vitamin C (mg)	48.0	45.0	49.0	16.0	62.0
Vitamin B ₁₂ (µg)	2.2	2.07	2.17	3.55	5.2
Folic Acid (µg)	116	130	114	87	218

* Modified from Wharton *et al.* (1984)** Gregory *et al.* (1990).

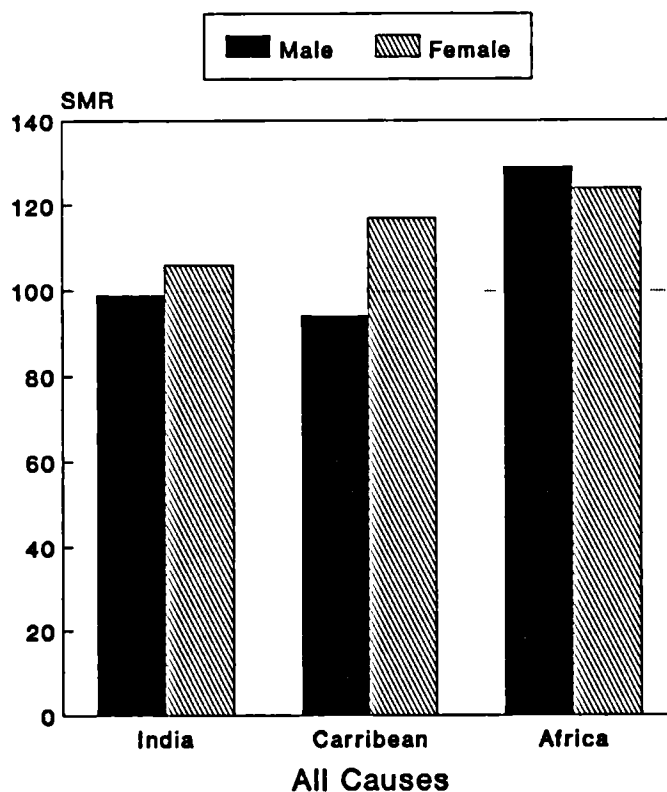
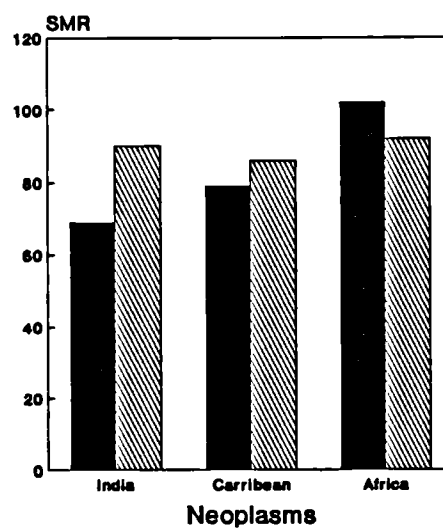
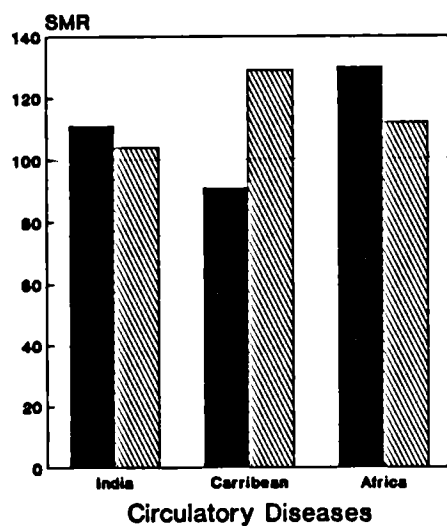


Figure 1:1 SMRs by country of birth, 1970-78

may have included migrants of Indian origin in other groups. Deaths due to neoplasms are lower in all ethnic groups except men born in African countries.

Ethnic Indians as a group showed a different pattern of mortality from that present in England and Wales (Adelstein and Marmot, 1989). A high incidence of tuberculosis is reflected in high mortality from infective and parasitic diseases. Among cancers, SMRs for liver cancer in men and women and buccal cavity cancer in men are high. Several other cancers are low. The low mortality from cancer of the trachea, bronchus and lung is consistent with low rates of smoking. The high incidence of buccal cancer is anomalous because of the low alcohol intake and low frequency of smoking in this group. Mortality is also high from diabetes and from suicides. The mortality from cirrhosis, particularly in women, is probably related to previous viral hepatitis rather than the result of alcohol abuse and may also be related to the high mortality from liver cancer.

Particularly striking is the high mortality from complications of pregnancy, childbirth and puerperium (343.3/million live births compared to 134.5 in 1970-1972), many of which are likely to be 'avoidable deaths' according to the Report on Confidential Enquiries into Maternal Deaths (DHSS, 1982).

An unexplained feature of mortality of immigrants from the Indian subcontinent is the flattening of the inverse relationship between social class and mortality seen as strongly in England and Wales (Adelstein and Marmot, 1989). There is an inverse association between social class and mortality from circulatory diseases and mortality among non-manual but not among manual classes.

Patterns of Mortality among Sub-Ethnic Groups from the Indian Sub-Continent

A proportional mortality analyses was carried out on the deaths in England and Wales in 1975-1977 of those born in India, Pakistan and Bangladesh (Balarajan *et al.*, 1984). They were further classified into sub-ethnic groups of Punjabis, Gujaratis, Muslims, South Indians etc. based on their surnames and forenames. Observed and expected deaths were aggregated for the entire period to calculate proportional mortality ratios (PMR) after standardising for age and sex (Table 1:4).

TABLE 1:4

Proportional mortality rates (number of deaths) for classified groups of diseases by ethnic group (Bengalis and others not listed owing to small numbers)

Cause of Death (ICD code)	Sex	Ethnic Group				Total
		Punjabis	Gujaratis	Moslems	Southerners	
<i>Infective & Parasitic Diseases (000-136)</i>	M	366* (25)	454* (15)	429* (37)	212 (1)	397* (79)
	F	539* (18)	638* (15)	571* (18)	1053* (2)	577* (53)
<i>Neoplasms (140-239)</i>	M	32* (66)	46* (58)	53* (138)	38* (6)	45* (282)
	F	42* (52)	44* (46)	61* (53)	63 (4)	48* (157)
<i>Endocrine, Nutritional & Metabolic Disease (240-279)</i>	M	254* (22)	292* (14)	180* (21)	267 (2)	224* (60)
	F	173 (10)	311* (15)	126 (6)	606* (2)	206* (33)
<i>Diseases of the Blood & Blood Forming Organs (280-289)</i>	M	210 (3)	109 (3)	213 (4)		160 (10)
	F	579* (7)	330* (3)	210 (2)		383* (12)
<i>Mental Disorders (290-315)</i>	M	254* (7)	78 (1)	28 (1)		112 (9)
	F			81 (1)		26 (1)
<i>Diseases of the Circulatory System (390-458)</i>	M	120* (445)	115* (260)	122* (551)	133 (38)	120* (1341)
	F	107 (113)	120* (111)	125* (88)	75 (4)	117* (326)
<i>Diseases of the Respiratory System (460-519)</i>	M	109 (80)	111 (49)	104 (86)	40 (2)	103 (220)
	F	157* (40)	112 (24)	97 (19)	70 (1)	124* (86)
<i>Diseases of the Digestive System (520-577)</i>	M	161* (35)	143 (18)	129 (37)	56 (1)	143* (96)
	F	108 (11)	107 (9)	171 (13)	175 (1)	124 (34)
<i>Diseases of the Genitourinary System (580-629)</i>	M	136 (12)	199* (10)	180* (20)	141 (1)	165* (44)
	F	328* (18)	339* (15)	257* (11)		278* (44)
<i>Accidents, Poisoning & Violence (E800-999)</i>	M	115 (97)	92 (35)	78* (99)	180* (11)	95 (251)
	F	132 (43)	112 (25)	77 (25)	142 (3)	106 (97)

Source: Balarajan *et al.* (1984); * $p < 0.05$.

Regional variations in disease patterns have been reported from India (Malhotra, 1967) and similar variation has been observed in the U.K. Cirrhosis of liver, cerebrovascular disease and mental disorders caused more deaths in Punjabi males. Deaths due to ischaemic heart disease were not significantly greater in Punjabis as opposed to other groups, but they had the lowest mortality ratios for cancer overall in both sexes. Lung cancer ratios were lower than in the Muslims but not as low as Gujaratis. Deaths due to diabetes were highest among Gujaratis as were deaths due to neoplasms of the buccal cavity and pharynx.

Muslims as one group have a common feature irrespective of their regional origins: they are all non-vegetarians. They exhibited the highest PMRs for cancer compared with the other groups, most appreciably for cancer of the lung or breast. Mortality due to cervical cancer was lowest in Muslim women which could be related to the higher incidence of circumcision in the males. Muslims also had the highest ratios for ischaemic heart disease.

Perinatal and Infant Mortality

The latest analysis published for 1984 shows that perinatal mortality and neonatal death rates have been consistently higher for mothers born in the New Commonwealth and Pakistan. Within these, mothers born in India and Bangladesh have shown a recent reduction in rates. There is, however, accumulating evidence from local investigations of considerable disparities in health experience between white and black populations as a whole at and around childbirth (Grimsley & Bhat, 1989), which may be related to possible causes of perinatal and infant mortality.

Post-neonatal mortality was highest in infants of mothers born in Pakistan (6.4) followed by infants of mothers born in the Caribbean (4.5), the U.K. and the Republic of Ireland (4.1). However, infants of mothers born in India (3.9), Africa (3.0) and Bangladesh (2.8) had a lower mortality than infants of UK born mothers (Balarajan, 1989). An important finding was low incidence of sudden infant death (cot death) in infants of Asian origin, which accounts for 42% of all post-neonatal deaths in the U.K. However, 30% of post-neonatal deaths in infants of Asian origin are due to congenital anomalies, which is double that seen in non-Asians. Genetic factors including consanguinity may play a role (Terry *et al.*, 1985).

1:8 Nutritional Problems and Nutritionally-Related Diseases

1:8:1 Low Birthweight

In a study of 2661 pregnancies by Studd *et al.* (1982) in Dulwich Hospital, there were no specific problems observed in Asian mothers. The incidence of anaemia, essential hypertension, pre-eclampsia, placental abruption and prematurity were all similar to that observed in mothers of Caucasian and Afro-Caribbean origin. Nevertheless, low birthweight is more common in babies born to mothers of Asian origin (11%) compared to those of U.K. (6.8%). Several studies have concluded that the babies of Asian mothers are lighter than those of European mothers of similar height and parity and they have anthropometric and biochemical evidence of pathological growth failure (Fosbrooke & Wharton, 1973; Wharton, 1981). However this is unlikely to be genetic as some improvement in environmental factors has resulted in a secular increase in birthweight in all Asian sub-groups (Clarson *et al.*, 1982). Furthermore, although parental consanguinity is associated with an increase in the number of low birthweight babies, overall effect on mean weight is small (Honeyman *et al.*, 1987).

There is evidence to suggest that undernutrition could be one of the environmental factors; firstly food customs and low nutrient intakes suggest that some mothers could experience nutritional stress (Eaton *et al.*, 1984); secondly, mothers laying down insufficient fat stores in the second trimester of pregnancy (i.e. showing no increase in triceps skinfold thickness in that period) had poorly grown babies (Bissenden *et al.*, 1981); thirdly, mothers with evidence of compromised energy balance benefitted from a protein energy supplement and gave birth to heavier babies (Viegas *et al.*, 1982a), whereas mothers who had put on sufficient fat did not benefit (Viegas *et al.*, 1982b) and indeed there may have been an adverse effect. In a study in an affluent population of Indians and Caucasians the mean birthweight to Hindus was 180 g lighter than that of Caucasians. Since most of the vegetarians were Hindus birthweight was compared between vegetarians, Hindu meat-eaters and Muslim meat-eaters. The major difference in average birthweight was between all Hindus (2951 g) and Muslims (3133 g) - a difference of 182 g. The authors (McFadyen *et al.*, 1984) concluded that the heavier Muslim babies could not be explained by differences in gestational age, parity, maternal weight or social class. Also vegetarianism does not appear to have a direct effect on birthweight as similar intakes of energy have been measured in non-vegetarians.

Sub-ethnic variation of birthweight observed in the Asian population cannot be further explained by energy intakes (Wharton *et al.*, 1984), despite lower energy intakes Muslim babies seem to be heavier at birth than Hindu babies. Low zinc and copper status in Asians has also been suggested to be the cause for slow interuterine growth, but studies have found no association (Campbell-Brown, 1985). Low birth-weights in Indians have also been observed in Singapore, where Chinese and Malay babies are heavier than Indian babies (McFadyen, 1985).

1:8:2 Growth

Distinct differences in linear growth, weight and fat deposition between children from different ethnic groups have been noted (Rona and Chinn, 1986; 1987) as part of a national surveillance study. Irrespective of social factors Indo-Pakistani children were lighter than other groups, but boys in this group had a higher triceps skinfold thickness in relation to National Study of Health and Growth (NSHG) standards than the girls. In the same study parents' weight and height were most highly associated with child's weight for height and triceps skinfolds. Small stature is a common parental concern especially among the Bangladeshis (Black, 1984). However, in many cases the parents were small and the child was growing at a normal rate. A longitudinal study by Warrington *et al.* (1988) of Asian and Caucasian children in the first two years showed a similar growth pattern as indicated by rates of change in body weight, body length and head circumference even when matched for sex and socio-economic status. However, compared to Tanner and Whitehouse UK growth standards (1965), both groups were below 50th centile but the Asians were smaller than Caucasians.

Concern has been raised how late weaning may limit the growth potential of a genetically small population. There is evidence of growth faltering and undernourishment around the time of weaning (Jivani, 1978) and indeed a slight reduction in mean growth between one and three years (Harris *et al.*, 1983).

Growth differences within the Asian sub-ethnic groups have also been reported. Punjabi children tended to remain above 90% of the Tanner 50th centile for body weight throughout the first two years (McNeil, 1985), with Bangladeshi children approximating to the 25th centile (Davies and Wheeler, 1989). Rona and Chinn (1986) reported less difference among sub-ethnic groups in weight for height, but there were some differences

by sex. Children of all Asian groups tended to be lighter for unit of height for age, Gujaratis being the shortest, on an average 3 cm shorter than Caucasians. Punjabi boys were above and Gujarati boys below the 50th centile of triceps skinfold while all groups of Asian girls were well below. Adolescent boys of Indo-Pakistani origin were reported to have larger triceps and sub-scapular skinfold compared to Caucasians and West Indian children (Rona & Chinn, 1987). However, British Asian children have been found to be taller than native Asians and this may be attributed to improved nutrition and a low incidence of disease (Warrington & Storey, 1988).

1:8:3 Nutritional Anaemias

Iron deficiency anaemia

Compared with the general population Asians have a higher incidence of iron deficiency (microcytic) anaemia, particularly vegetarians (Ehrhardt, 1986). However, it is not usual in Caucasian vegetarians (Sanders *et al.*, 1978). Infants and pregnant women are most susceptible, incidence being highest in those who rely on rice rather than wheat (Goel *et al.*, 1978). Iron deficiency has been observed in infants of Asian origin since 1960 (Davis *et al.*, 1960). A prevalence as high as 40% (haemoglobin < 110 g/dl) was found in Asian infants aged 22 months (Grindulis *et al.*, 1986). Iron deficiency anaemia in infancy is associated with late weaning practices and lack of iron supplementation leading to comparatively low haemoglobin concentrations and serum ferritin levels (Ehrhardt, 1986). It has also been claimed that children of parents from the Indian sub-continent are more at risk of iron deficiency than Caucasians (Hussain & Wadsworth, 1967). The cause of anaemia was more recently assessed in Punjabi females over 11 years of age, 32% were anaemic mostly due to iron deficiency indicated by serum iron concentration below 10 mmol/l (Britt *et al.*, 1983).

Megaloblastic Anaemia

In Asians, the incidence of megaloblastic anaemia resulting from combined vitamin B₁₂ and folate deficiency is three times the national average, seen mainly in vegetarians (Mathews & Wood, 1984; Chanarin *et al.*, 1985). Vitamin B₁₂ deficiency was predominantly seen in Gujarati women, although some had associated folate deficiency. Megaloblastic anaemia may be precipitated during pregnancy and babies may also be prone to develop severe vitamin B₁₂ deficiency, particularly if breast fed with milk of low vitamin B₁₂ content (Roberts *et al.*, 1973). Chanarin & Stephenson (1988)

hypothesised that the low dietary intake of vitamin B₁₂ in vegetarian Gujaratis might impair killing of bacilli, particularly tubercle by macrophages whose phagocytosis is cobalamin dependent and found that active tuberculosis infection coexisted in 12% Hindus with megaloblastic anaemia. If proven, some of the excess of tuberculosis in Indians could be explained by a dietary deficiency of vitamin B₁₂.

1:8:4 Diabetes Mellitus

Non-insulin dependent diabetes (NIDDM) has been long known to ancient physicians in India and its prevalence has been well recognised in the middle-aged, affluent and obese (McCay *et al.*, 1916; Mukherjee, 1973). Excess prevalence of diabetes has been consistently reported in Indians living overseas - from Malaysia, Trinidad, South Africa, Singapore (West & Kalbfleisch, 1966; Poon-King *et al.*, 1968; Marine *et al.*, 1969; Cheah *et al.*, 1979). In Britain, standardized and proportional mortality ratios for diabetes have been twice the national average for people born in the Indian subcontinent (Balarajan *et al.*, 1984). Within a generation of migration to Britain, the diabetes epidemic among Indian or East African born Asians became obvious, confirmed by reports from Southall, West London (mainly Punjabis), North West London, Leicester (both mainly Gujaratis), Coventry and in Bangladeshis in East London (Mather and Keen, 1985; McKeigue *et al.*, 1988; Simmons *et al.*, 1989).

Mather and Keen (1985) compared prevalence of diabetes in Southall Punjabis with that in a relatively affluent suburb of Delhi and found dramatic differences in age-specific rates between Indians and Caucasians in Southall, but their close similarity with Indians in Delhi. The age-specific rate ratio being four to eight times that for Southall Caucasians in both migrants and Punjabis in Delhi, with an unusually high prevalence in the age group of 40-64 (Verma *et al.*, 1985).

Impaired glucose tolerance was observed in 50% Gujarati men and women tested in North West London (Cruikshank, 1989). However, these are preliminary results which also showed similarly high glucose intolerance in Caucasians, but the total numbers of known and newly-diagnosed diabetics among Gujaratis was relatively higher. These prevalence rates were associated with markedly increased insulin secretion for given levels of glucose in Gujaratis compared with whites. Twice as high serum concentrations

of insulin have also been reported in Bangladeshis after a glucose load compared to non-Asians (McKeigue *et al.*, 1988).

From the elevated insulin-glucose ratio and a high diabetes prevalence it may be inferred that insulin resistance could be present in Indians generally (McKeigue *et al.*, 1989). Insulin resistance is strongly associated with obesity and particularly with the relative proportion of body fat deposited intra-abdominally. There is no data on the extent to which the pattern of adipose tissue distribution differs between Indians and others. However, the measure of obesity such as Body Mass Index (BMI) is not higher in Indians compared to other groups (McKeigue *et al.*, 1988; Miller *et al.*, 1988).

Insulin dependent diabetes (IDDM) is relatively uncommon and those Asians who are receiving insulin therapy are not really insulin dependent cases but rather are those in whom dietary caloric restriction or oral hypoglycaemic agents have been unsuccessful (Mather and Keen, 1985).

1:8:5 Coronary Heart Disease

An early study in Singapore (Danaraj *et al.*, 1959) found a higher incidence of CHD in Indians which was also reported from East and South Africa, Fiji and the Caribbean (Shaper & Jones, 1959; Walker, 1963; Sorokin, 1973; Miller *et al.*, 1982). The risk in countries of origin is unclear, but high rates have been observed in urban regions (McKeigue *et al.*, 1989). An increased risk of CHD appears to apply universally to migrant subgroups of Indian subcontinent origin despite variable socioeconomic prosperity, dietary habits and duration of domicile in their adopted country (Hughes and Cruikshank, 1989).

Britain has currently the worst record worldwide without the decline reported in the U.S.A. and elsewhere (Doll, 1987). Study of mortality statistics for England and Wales (Marmot *et al.*, 1984) has shown that CHD mortality in Indians is 20% higher than the national average. An increased incidence of myocardial infarction was first reported by Tunstall-Pedoe *et al.* in 1975 among predominantly Bangladeshi immigrants living in deprived areas of East London. This excess representing between 13-20%, is seen in both sexes and in all ages, but with increased frequency in the younger age groups of 35-54 in men and a lower frequency in the older age group (55-64) (Beevers

and Cruikshank, 1981). In the same study an increase was observed in post-menopausal women.

Among hospital admissions in Leicester with ethnic group classified by surname, the odds ratio for myocardial infarction was 2.2 for Asians compared with others and was higher in the 25-44 year age group (Donaldson and Taylor, 1983). However, these studies are based on admissions into hospitals which may be a result of variable response to chest pain in different ethnic groups, but this does not explain the excess of death rate from CHD observed in Indians. A recent study of Asians in five different London boroughs (McKeigue and Marmot, 1988) has confirmed a mortality from CHD 50% higher than the national average. The communities were very diverse in terms of religions, countries of origin, economic status, smoking and dietary habits, but it has been suggested that one factor they may have in common is disturbed insulin metabolism (McKeigue *et al.*, 1988).

Coronary angiographic studies have also revealed that coronary artery disease in Asians was quantitatively more severe with extensive coronary artery damage than in Caucasians, irrespective of risk factors such as smoking and cholesterol concentrations which were lower in the Asians (Lowry *et al.*, 1984). It is interesting to note that in the same study, in Asians with proven coronary disease, as many as 35% had normal ECGs (electro-cardiograms). A significant relationship between the extent of atheroma and cholesterol concentration was found in Asians and not in Caucasians, although the Asians had significantly lower cholesterol levels (Hughes *et al.*, 1990).

Risk Factors Associated with CHD

1. Smoking. Most studies have shown that smoking rates are lower in Asian men and rarely seen in Asian women (Balarajan and Yuen, 1986), but a significantly higher rate has been observed in Bangladeshis (McKeigue *et al.*, 1988) compared to other sub-ethnic groups. However, this potent and reversible risk factor cannot wholly explain the higher incidence of CHD in this group but when presented along with other risk factors it may have a synergistic effect on mortality and morbidity.

2. Hypertension Hypertension is common in urban and rural populations in the Indian sub-continent (Gupta *et al.*, 1979; Islam *et al.*, 1983). Migrant Punjabi

women in Southall showed 23% age-adjusted hypertension compared with 1.4% in the native Punjabi women. There was a significant increase in blood pressures with age in both groups but migrants' pressures were higher at almost all ages. However, Southall Punjabis were on average 10 kg heavier than their counterparts and obesity was strongly correlated with blood pressure elevation even after adjusting for age.

Comparative studies in the UK have not found higher blood pressures in Asians when compared to Europeans. In North West London mean blood pressures were similar in Indians and Caucasians and in East London mean systolic (but not diastolic) pressures were 10 mmHg lower in Bangladeshi men and women than in Europeans (Miller *et al.*, 1988; McKeigue *et al.*, 1988). Mean blood pressures by age decade were not significantly different among the Caucasian, Asian and West Indian male employees in a Birmingham factory. However, West Indian women had significantly raised blood pressure which was related to higher body mass index (Cruikshank *et al.*, 1985). Hence, hypertension may not be a significant factor in the excess of CHD in Asians.

3. Plasma Lipoproteins No consistent excess of hyperlipidaemias has been reported in Asians. On a population basis, the risk of CHD rises progressively with increases in serum total cholesterol from 3.89 mmol/l (150 mg/dl). Observational studies suggest that one population with an average total cholesterol level 10% lower than that of another will have one-third less CHD, and a 30% difference in total cholesterol predicts a four-fold difference in CHD (WHO, 1990). Values higher than 5.2 mmol/l have been reported for urban groups in various parts of India but not in middle and lower income groups. However, such comparisons are difficult to interpret due to varied sampling procedures, laboratory techniques etc. (McKeigue, 1989).

Mean cholesterol concentrations tend to be higher in Indians living overseas compared to Indians in the home country, but no study has shown concentrations similar or above that of the British men, nor higher than other ethnic groups with low incidence of CHD. In contrast to local mortality data, mean cholesterol levels in Asians in North West London and East London were found to be significantly lower than Europeans (Miller, 1988; McKeigue, 1988). Significant differences in plasma cholesterol fractions have been observed. Studies both in the U.K. and abroad have reported lower HDL cholesterol concentrations in Indians compared to Europeans. In neither Gujaratis nor

Bangladeshis living in London was there a sex difference in HDL cholesterol and a similar lack of differences between sexes has been reported in studies conducted in India (Gandhi, 1982; Shanmugasundaram, 1983), which is in contrast to the usual finding in European communities at high CHD risk (Miller and Miller, 1975).

Triglyceride levels in plasma have been reported to be lower in Indians compared to Caucasians, but not in West Indians (Miller *et al.*, 1988). However, Hughes *et al.* (1990) in a case control study found significantly higher triglyceride levels in both groups of Asian controls and patients who survived myocardial infarction, compared to Caucasian patients and controls. It is sometimes difficult to interpret triglyceride concentrations, because it is essential to ensure that a fasting blood sample is collected for analysis. In Bangladeshis 50% higher levels were observed after a glucose load (McKeigue *et al.*, 1988).

4. Haemostasis Fibrinogen and factor VII coagulant activity (VIIC) levels have been found to predict CHD. No significant differences in fibrinogen concentrations and factor VIIC were observed between Indians (Gujaratis) and Caucasians (Miller *et al.*, 1988). Bangladeshi men and women had similar fibrinogen levels to Europeans, but factor VIIC was lower in Bangladeshi than Caucasian men (McKeigue *et al.*, 1988). Fibrinolytic activity was found to be slower in Indian men and women compared to West Indians, with Europeans having an intermediate value, although higher levels were observed in all women due to the influence of circulating oestrogens (Cruikshank, 1989). In contrast to clotting factors, significantly higher platelet count (15%) and lower mean platelet volume (5%) were reported in Indians. However, no differences in platelet aggregation in Indians have been reported so far.

5. Diet Dietary fat in particular does not afford a satisfactory explanation to increased rate of CHD in Asians. In two studies (McKeigue *et al.*, 1985; Miller *et al.*, 1988), one using 7-day household food inventories of all food bought into the home in 184 Asian households in North West London and the other 5-day weighed intakes in a sample of the same population, Gujaratis were found to have low dietary saturated fat intakes and high polyunsaturated fat intakes compared with the British average. Total fat intake was similar to the levels in the British population, but P:S ratio was higher in the Asians. Intakes of n-3 levels of fatty acids of marine origin, which have been suggested

to afford protection against atherosclerosis were low, but higher intakes have been reported in Muslim Bangladeshis in East London, reflecting consumption of fish in the diet (McKeigue *et al.*, 1988). Cholesterol oxides in ghee (clarified butter) have been suggested as a possible cause of CHD in Asians based on animal studies which found them to be atherogenic, but it needs further investigation. However, ghee consumption is not uniform in all the subethnic groups of Asians as is the prevalence of CHD.

Effects of dietary fat, fatty acids, cholesterol and dietary fibre on coronary heart disease are discussed in detail in Part II of this chapter.

6. Diabetes The one risk factor for CHD which is more prevalent in people of Indian origin is diabetes mellitus, particularly non-insulin dependent diabetes. Higher prevalence of diabetes may account for the excess of CHD in Asians but this needs to be confirmed by further studies as Afro-Caribbeans who are less susceptible to heart disease also have a high prevalence of diabetes. Approximately 13% of excess of CHD could be accounted for by hyperglycaemia in Indians in Trinidad (Miller *et al.*, 1988). The hypothesis that ineffective peripheral insulin action is a common factor in the Asian propensity to both diabetes and CHD has been proposed, but the prevalence of low incidence of CHD in Afro-Caribbeans accompanied by high insulin/glucose values does not support it. However, disturbances in insulin action may be present long before overt CHD (Cruikshank, 1989). Hyperinsulinaemia has been a common finding in Indians living overseas.

7. Other Factors Some reports place emphasis on the role of stress in the cause of CHD in Indians (Coronary Prevention Group, 1986). Asians may be subject to higher levels of stress because of poor social environment, racism and the psychological trauma of migration. However, other ethnic minority groups are subject to similar stresses but they do not exhibit excess CHD mortality rates. Genetic factors do not explain the excess of CHD in Asians either, groups of Indians living overseas originate from different parts of the subcontinent. Moreover, gene frequencies of Class I and Class II antigens are similar to those in Europeans and the frequency profile in young Indian men with history of myocardial infarction has not been found to be distinctive (Mehra *et al.*, 1986).

1:8.6. Cancer

Neoplasia in general appears to be less common worldwide in Asians compared to Caucasians (WHO, 1982). In the few studies that are available this pattern seems to persist in Asians who have migrated to the UK (Donaldson and Clayton, 1984; Potter *et al.*, 1984). An Asian patient is therefore just under half as likely to be discharged from hospital with a diagnosis of malignancy as a Caucasian patient (Donaldson and Taylor, 1983). Changes in cancer incidence in migrant populations are of great interest because of the clues they give as to the causes of cancer, but as yet there is little information on changes in cancer incidence over the last twenty years in the Asian population in the UK. Pattern of disease at present reflects that of the populations of origin, although in many instances incidence is even lower because of the 'healthy cohort effect' and the relative youth of migrants (Sturman and Beevers, 1990).

From published studies (Matheson *et al.*, 1985) it appears that the incidence of lung cancer is about two-thirds that expected, which is consistent with low frequency of smoking; the incidence of colo-rectal cancer is about one-half to one-eight that expected and the incidence of breast cancer is about one-half of that expected. However, the numbers of actual cases involved in these studies are small. Stomach, testis and skin malignancies are also significantly less common in Asians. In contrast to the above cancers, cases of carcinoma of the cervix in Asian women, were found to be about one and a half to four times in excess when compared to the indigenous population, an incidence rate much closer to that found in Bombay.

Cervical cancer is by far the commonest malignancy in women in the Indian subcontinent, with a crude incidence rate in 1975 of 24 in every 100,000 women (Parkin *et al.*, 1984). This is one and a half times the current incidence rate in England and Wales. A study of immigrants in England and Wales, based on census data and death certification (Marmot, 1984) found a SMR of 115 for cervical cancer in those from the Indian subcontinent. But, when women of British descent were excluded, the PMR for Indian immigrants is 69. A further study of causes of deaths in immigrants from the Indian subcontinent showed that Muslims have a particularly low PMR (19) for cervical cancer.

A smaller study by Donaldson and Clayton (1984), comparing cancer registrations in Asians and non-Asians over seven years in the Leicestershire population (62% Hindu, 18% Muslim, 17% Sikh), estimated a standardized registration value of 1.2 for cervical cancer. Many of these Asian families studied emigrated from Africa where they had been resident for over a hundred years. A similar excess of cervical cancer was reported in the Indian population in Natal, South Africa (77% Hindu and 16% Muslim) (Schonland and Bradshaw, 1968). Lack of knowledge or awareness leading to poor uptake of cervical cytology by all religious groups (approximately half that of the indigenous population) has been suggested to be associated with higher incidence of cancer of the cervix in the Asians (McAvoy and Raza, 1988).

Breast Cancer Age-adjusted incidence and mortality rates for breast cancer are the highest in Western Europe, the U.S.A., Canada, Australia and New Zealand; intermediate in Eastern and Southern Europe, and lowest in Asia, Latin America and Africa (Waterhouse *et al.*, 1976). The incidence in Asian countries, with the exception of India, increases to a perimenopausal plateau but declines progressively thereafter, in contrast to the continuing increase among older women in Western countries. This suggests that environmental factors play a more important role in the aetiology of post-menopausal breast cancer, whereas genetic, endocrinological and other endogenous factors strongly influence the pre-menopausal disease (Petrakis *et al.*, 1982). Daughters of European immigrants to the U.S.A. reach the incidence rates of daughters of American-born parents within one generation; whereas Japanese women appear to require more than one generation, whether this is the case with Asian immigrants to Britain is not known due to a lack of ethnic national figures for breast cancer in Britain (McAvoy, 1990).

While breast cancer is less frequent in Asians than in Caucasians, the mean age of presentation is said to be twelve years younger than the mean age in Caucasians and the disease is more advanced. Although retrospectively out of 883 women with breast cancer only 1.9% were of Asian origin compared to 3.4% of Afro-Caribbean origin in Birmingham (Potter *et al.*, 1983, 1984). This may be a reflection of the younger age distribution of the population, but could also be related to their higher parity and lower rate of breast feeding.

1:8.7. Vitamin D and Calcium Deficiency - Rickets and Osteomalacia

In the UK rickets and osteomalacia in the past had been confined to low socio-economic groups and the elderly, but increased frequency in individuals of Indian subcontinent origin have been documented since 1962, first described in Pakistani Muslims in Glasgow (Dunnigan *et al.*, 1962). Asian rickets and osteomalacia differ from vitamin D deficiency in the Caucasian population, being found not only in infancy and the elderly but throughout childhood, adolescence and adults, especially in women (Robertson *et al.*, 1982). Holmes *et al.* (1973) in a formal assessment of prevalence of nutritional rickets and osteomalacia, found 30% of Asian adults and children in Rochdale had clinical rickets compared with 4% of Caucasian children, but comparable data for adults were not available. A later survey (Stephens *et al.*, 1982) in Muslim Asian children and not adults showed improvement in biochemical variables of vitamin D deficiency. Vitamin D levels below 12.5 nmols/l occurred in 33% of girls and 22% boys, in 35% of Asian men and 33% of Asian women. Mean plasma concentrations in both children and adults were less than in Caucasian controls.

Daily dietary vitamin D of less than 50 IU was recorded in 32% of Asian and 24% of Chinese but in only 9% of white and 7% of African children (Goel, 1976). In Bradford, Ford *et al.* (1976) conducted a retrospective case record survey of hospital admissions due to rickets between 1969 and 1972. Rickets was solely confined to 23 Asians (out of 156) in the 9-16 year age group during the four years and in 22 Asians and 10 Caucasian children aged 0-3 years. In the random sample abnormalities of calcium, phosphate or alkaline phosphatase were noted in 79 Asians, 9 West Indians, but in no Caucasians. 25-Hydroxycholecalciferol (25(OH)D) levels were lowest in Asians with 41% having very low levels (below 3.8 ng/ml). Significantly lower concentrations have also been reported in studies from Edinburgh and Bradford (O'Hare *et al.*, 1984; Grindulis *et al.*, 1986).

Reduced concentrations of 25(OH)D representing true vitamin D deficiency may be implicated in 'Asian' osteomalacia. Plasma levels tend to fall in Asians during residence in the U.K. (Preece *et al.*, 1975), but are normal on arrival (Stamp, 1980) and in the country of origin (Hodgkin *et al.*, 1973). The increased skin pigmentation of Asians as a possible cause of deficiency is unlikely as Afro-Caribbeans with darker skins do not have the same problems. However, Indians, Pakistanis and Caucasians have been

reported to have similar capacity to produce vitamin D in response to ultraviolet irradiation (Lo *et al.*, 1986). There is some evidence that rachitic children have less outdoor exposure than those without any signs of the disease (Stamp and Round, 1974). Lower dietary vitamin D in vegetarians, high fibre intake and low meat diets appear the most likely causes of vitamin D deficiency (Dunnigan *et al.*, 1982). Anti-nutrients, such as phytic acid contributed by dietary fibre, particularly in chapattis (unleavened breads), appears to be implicated in 'Asian rickets', and reducing dietary phytic acid promoted healing (Ford *et al.*, 1972; Wills *et al.*, 1972). Heath (1983) proposed that high phytate diets bind vitamin D in the gut resulting in its excretion and thus greater vitamin requirements.

Hindus, who are predominantly vegetarian, have been found to have lowest levels of 25(OH)D when compared to Muslims and Sikhs, although no comparable Caucasian control group was studied, their mean 25(OH)D level of 15.7 nmol/l was lower than that considered normal in Caucasians (Hunt, 1976). In a comparison of vitamin D status of 60 healthy adult Hindu couples with 48 healthy Caucasian controls, 22% of Hindus had levels of 25(OH)D below 10 nmols/l compared with none of the Caucasians. Sex differences were not observed but marked similarities of 25(OH)D concentrations between couples were found. The duration of time in the UK and vegetarianism had little effect on vitamin D status with Punjabis having lower levels than Gujaratis (Shaunak *et al.*, 1985).

The problem of reducing the risk of osteomalacia among Asian women is likely to be more difficult than rickets among infants and children, but for those Asians at risk, the importance of exposure to sunlight, generally nutritious diet and perhaps the use of supplements must be emphasized (DHSS, 1980).

1:9 Vegetarianism in Caucasians

There has been a considerable increase in interest in vegetarianism in recent years. Vegetarians now represent 3.7% of the adult population (Realeat Survey, 1990), a considerable increase since the survey began in 1984, when vegetarians registered 2.1%. Those avoiding red meat, not totally distancing from animal foods, represent 6.3% of the adult population, the main reason for this was health cited by four in ten of the category. Pure vegetarians are twice as likely to be found in the higher social grades but there is

less social difference in the numbers who are avoiding red meat. Women are far more likely to be vegetarians than men, although there has been an increase in vegetarianism among men during the 1980s.

Most of those becoming vegetarians or cutting red meat out of their diet seem to be doing so in the South of England, mainly for health reasons. Other reasons being: an abhorrence of animal suffering; objection to intensive animal farming methods; an association of vegetarianism with purity of thought; a belief that a vegetarian diet is more natural and wholesome than the typical Western diet and therefore healthier. Another reason that has been given more recently is that a vegetarian diet is more economical in the context of the limited world food resources (Sanders, 1988).

Vegetarianism today seems to be more popular with the younger age group (16-34 years) and is often associated with movements concerned with the environment and other altruistic causes. Vegetarianism may not be confined to diet alone but seems to encompass a change in lifestyle; for example, vegans do not use animal products such as woollen clothes, fur coats or leather shoes, or use cosmetics that contain animal products or that have been tested on laboratory animals.

1:9.1 Types of Vegetarian Diets

Vegetarian patterns differ widely, often based on the reasons for which they have been adopted. All vegetarians exclude meat and most will also exclude fish; an ovo-vegetarian diet includes eggs, thus an ovo-lactovegetarian diet includes both eggs and milk products; a vegan or strict vegetarian diet contains no food of animal origin; a fruitarian diet, which is the most extreme or ultimate form, consists only of raw fruit, nuts and berries. A vegetarian diet is usually adopted gradually, firstly by avoiding red meat, followed by poultry and fish. Some progress on to become vegans excluding eggs, milk products and all animal products, even honey, from their diet.

In the past, vegetarian diets could be restricted in variety in foods and were perceived by many as unattractive and monotonous. Presently, due to a vast improvement in food technology, transportation of foods, a wide variety of fruits and vegetables are available through all seasons, so that vegetarian diets can be varied and attractive. Moreover, the commercial food industry, in response to the increased appeal

for vegetarianism, has made a variety of ready-to-eat vegetarian foods easily accessible in most supermarkets.

Household food consumption in non-Asian vegetarians was compared by Bull and Barber (1984) from the National Food Survey 1979-1982 (Table 1:1). Consumption of milk and milk products was lower than all households but cheese seemed to be more popular, probably to compensate for the absence of meat and fish in the diet. Non-Asian vegetarians consumed less butter, other fats, sugar and preserves, beverages and bread. However, consumption of flour, rice, other cereal products and margarine was higher than the national average. Although both were vegetarians, striking differences were found in the food consumption pattern of Asians and non-Asians. Asian vegetarians consumed specifically greater amounts of milk products, butter, other fats, vegetables and cereal products other than bread. Cheese, eggs, margarine and beverages appeared to be less popular with the Asians compared to non-Asian vegetarians.

1:9.2 Nutritional Adequacy of Vegetarian Diets

The specific nutritional question which vegetarianism raises concerns the nutrients that are normally provided by food of animal origin and how these can be replaced. In a mixed diet animal foods form important sources of iron, calcium, zinc, vitamins D and B₁₂ which may be compromised in a vegetarian diet. Most vegetarian diets have fared well when they are compared in their adequacy, variety, balance and moderation with the dietary patterns of non-vegetarians and with current dietary recommendations (Carlson *et al.*, 1985; Sanders, 1988; Dwyer, 1988). Most studies of vegans and vegetarians show that they have similar, or slightly lower, intakes of dietary energy compared with non-vegetarians (Ellis and Mumford, 1967; Bull and Barber, 1984). Both vegetarians and vegans consume less fat and more carbohydrate than non-vegetarians. Bull and Barber (1984) compared nutrient intake of non-Asian vegetarian households with the national average and found that intakes of vegetarians were lower in respect of all nutrients except calcium, vitamin C and folic acid. The positive aspects being the levels of dietary energy from protein, fat and carbohydrate were 11%, 38% and 51% respectively, thus conforming quite closely to the current recommendations (WHO, 1990).

One major difference between Caucasian vegetarians and omnivores is their preference for unrefined foods, particularly wholegrain cereals. Besides ensuring an

adequate intake of most of the vitamins with the notable exception of vitamin B₁₂, these foods provide large amounts of dietary fibre. Consequently, vegetarian diets, especially vegan diets, contain far more dietary fibre than those of non-vegetarians which has led to concern about its effect on mineral absorption - especially iron, zinc, calcium, etc. (Freeland-Graves *et al.*, 1980; Abdulla, 1981; Anderson *et al.*, 1981). Problems of nutrient adequacy only arise when vegetarian diets are monotonous and extremely restricted, especially if avoidances are extensive and exclude many foods, not only animal foods (Dwyer, 1988).

During periods of physiological stress and accelerated growth, there may be a risk of nutrient deficiencies. Problems of dietary inadequacy are more likely to occur in children than in adults, as their requirements relative to body weight are greater and they are unable to exert the same degree of control over what they eat with adults (Sanders, 1988). Severe malnutrition has been reported in children fed inappropriate vegan diets (Roberts *et al.*, 1979; Ward *et al.*, 1982). However, children can be successfully reared on vegan diets, provided considerable care is taken to avoid the major hazards of excessive bulk, and of vitamin B₁₂ and vitamin D deficiency (Sanders and Purves, 1981). Vegetarian diets that contain reasonable amounts of milk products and eggs are less likely to be inadequate.

1:9.3 Health of Caucasian Vegetarians

Clinical studies on the general health of Caucasian vegetarians and vegans have failed to show that they are less healthy than non-vegetarians (Sanders *et al.*, 1978; Haines *et al.*, 1980; Gear *et al.*, 1980; Abdulla *et al.*, 1981). There is no clear evidence of a difference in all-cause mortality between vegetarians and non-vegetarians. Burr & Sweetman (1982), in a prospective study of the users of health food shops in the U.K., found no significant difference in all-cause mortality between vegetarians and non-vegetarians, but the non-vegetarian sample was atypical of the general population both with respect to dietary practices, social class and smoking habits. Total mortality rates were lower than expected in both the vegetarians and the non-vegetarians, but there was a tendency for mortality from coronary heart disease to be lower among the vegetarians. Vegetarian Seventh-Day Adventists in the U.S.A. had lower age-specific mortality compared to omnivores from the same sect. Mortality from coronary heart disease was lower and mortality from cancer was no higher than in the general population (Kahn *et*

al., 1984). However, abstinence from smoking and alcohol was clearly associated with the lower mortality from cancers of the respiratory tract, head, neck and bladder (Lemon *et al.*, 1964, 1966). Adoption of such a healthy lifestyle may explain their lower overall mortality.

Both vegetarians, and particularly vegans, tend to be lighter in weight than non-vegetarians and this is related to a lower proportion of body fat (Sanders, 1983). Studies of both vegetarian and vegan children (Widdowson, 1948; Sanders and Purves, 1981; Jacobs and Dwyer, 1988) show that their growth and development is normal. However, there is a tendency for both vegetarian and vegan children to be smaller in stature and lighter in weight when compared with standards.

Nutritional Anaemias Iron intakes in most Caucasian vegetarians and vegans are usually high (Ellis & Mumford, 1967), although iron from plant foods is generally poorly absorbed compared with that from animal foods. These intakes could be regarded as adequate as haematological studies of Caucasian vegetarians have generally shown that they have normal haemoglobin concentrations (Armstrong *et al.*, 1974; Sanders *et al.*, 1978; Gear *et al.*, 1980). High consumption of fruits, vegetables and use of nutritional supplements by the vegetarians which enhance their intake of vitamin C may ameliorate the deleterious effects of dietary fibre on bio-availability of non-haem iron.

Vegetarians who consume eggs and milk regularly are likely to have sufficient intakes of vitamin B₁₂ but vegans may have low intakes (Sanders, 1988). Reports of megaloblastic anaemia have generally been confined to Asian vegetarians and vegans (Campbell *et al.*, 1982; Britt *et al.*, 1983). However, high folic acid intakes in vegetarians may be masking the manifestation of vitamin B₁₂ deficiency in those with inadequate intake of vitamin B₁₂ (Dwyer, 1988).

Calcium and Vitamin D Nutriture Calcium intakes in lacto-vegetarians tend to be similar or greater than in non-vegetarians (Ellis and Mumford, 1967), but lower in vegans owing to the absence of milk products from the diet (Sanders and Purves, 1981; Roshanai, 1983). Risks to calcium nutriture among vegans arise because of their low calcium intakes and the presence of several inhibitors of calcium absorption such as

oxalic acid, phytates, dietary fibre etc. in plant foods. The urinary excretion of calcium was reported to be very much lower in vegans than in non-vegetarians (Abdulla *et al.*, 1981) which would imply either that they are absorbing less calcium or that they are adapted to their low intakes.

Lower concentrations of serum calcium have also been found in vegetarians compared to controls (Ellis *et al.*, 1975). However, calcium nutritional status is rarely compromised if vitamin D status is satisfactory. Unlike in Asians, rickets and osteomalacia are uncommon in vegans, possibly because of adequate exposure to sunlight and most vegetable margarines are fortified with ergocalciferol which is acceptable to many vegans (Sanders, 1983). Vitamin D deficiency rickets have been reported in children fed on vegan diets and in infants who have been breast-fed for prolonged periods (Dwyer *et al.*, 1979; Ward *et al.*, 1982) and fail to supplement their diets with vitamin D.

Differences in the prevalence of osteoporosis between vegetarians and non-vegetarians are not well documented. Seventh-Day Adventist lacto-vegetarian women have been reported to have less bone loss after age of 60 than do omnivores (Marsh *et al.*, 1980), but no such difference was found in Seventh-Day Adventist males (Marsh *et al.*, 1983). However, bone densities between vegetarians and non-vegetarians were found to be similar (Ellis *et al.*, 1975). The lower rate of demineralisation of the bone in vegetarian women is unrelated to their calcium intake or initial bone density and appears to be more related to a high calcium : phosphorus ratio in the diet. Alternative explanations include a different effect of vegetarian diets on oestrogen metabolism or that vegetarians are physically more active than non-vegetarians.

Diabetes Mellitus Diabetes mellitus is only half as likely to be the underlying cause of death among Seventh-Day Adventists as in the American population as a whole (Snowden and Phillips, 1985). There may be a lower incidence of NIDDMs in vegetarians because they are leaner than non-vegetarians and hyperinsulinism associated with obesity and lowered tissue sensitivity to insulin may not be present. Other aspects of vegetarian diets such as high intake of complex carbohydrates, dietary fibre and generally low energy density of the diet could lead to improved glucose tolerance and increased insulin sensitivity (Munoz, 1984; Crapo, 1985). Popularity and prolonged breast-feeding among the vegetarians may be beneficial to decrease rates of insulin

dependent diabetes. An inverse correlation between the incidence of insulin-dependent diabetes mellitus in childhood and incidence and duration of breast-feeding was demonstrated among non-vegetarians (Borch-Johnson *et al.*, 1984).

Coronary Heart Disease Mortality and morbidity from coronary heart disease are lower in vegetarians than in non-vegetarians (West and Hayes, 1968; Phillips *et al.*, 1978; Burr and Sweetman, 1982). A proportionate mortality study based on the membership of the Vegetarian Society in the U.K. failed to show any difference in the number of deaths attributed to cardiovascular disease (Kinlen *et al.*, 1983). However, Burr and Butland (1988), in a prospective study of British vegetarians, found significantly lower mortality from ischaemic heart disease than in non-vegetarians; the differences were especially marked among men and they concluded that vegetarianism might confer some protection against ischaemic heart disease. It is important to consider the effect of vegetarianism on the risk factors to coronary heart disease.

The fat intake of vegans is devoid of cholesterol and is low in saturated fat but the saturated fat intake of vegetarians will depend almost entirely upon how much dairy products and eggs they consume. Plasma cholesterol concentrations of vegans are much lower than either vegetarians or non-vegetarians and are similar to the levels seen in populations where atherosclerosis is rare (Harding and Stare, 1954; Burslem *et al.*, 1978; Sanders *et al.*, 1978; Roshanai and Sanders, 1984), while those of vegetarians are similar or intermediate between those of vegans and non-vegetarians (Burr *et al.*, 1981; Thorogood *et al.*, 1987).

British Caucasian vegetarians were found to have similar or slightly lower plasma cholesterol concentrations compared to the non-vegetarians (Gear *et al.*, 1980; Burr *et al.*, 1981), but the ratio of HDL : LDL cholesterol was greater in the vegetarians. Roshanai (1983) reported much lower LDL cholesterol concentrations in British vegans than non-vegetarians but HDL cholesterol concentration was found to be similar. However, lower levels of both HDL and LDL cholesterol have been reported in vegans and vegetarians compared to controls (Sacks *et al.*, 1975; Burslem *et al.*, 1978). Nevertheless, differences between LDL cholesterol were far more marked than those between HDL cholesterol.

Lower levels of certain clotting factors (II, V, VII and X) and higher levels of anti-thrombin III and fibrinolytic activity have been reported in vegans and vegetarians (Haines *et al.*, 1980). These findings are of significance as high levels of LDL cholesterol and of clotting factor VII are independent risk factors for coronary disease. It would be predicted that vegans should be less prone to both atherosclerosis and coronary heart disease than meat eaters or ovo-lacto-vegetarians, as observed in male vegan Seventh-Day Adventists compared to lactovegetarian and omnivorous Adventists (Phillips *et al.*, 1978).

Most, but not all, studies have found lower systolic and diastolic blood pressure in Caucasian vegetarians than in the general population (Sacks *et al.*, 1974; Armstrong *et al.*, 1979; Haines *et al.*, 1980). The addition of meat to a vegetarian diet does not lead to an increase in blood pressure but the change from meat eating to a vegetarian diet resulted in a modest fall in both systolic and diastolic pressure (Margetts, 1986). The reduction in blood pressure was attributed to the increased potassium : sodium ratio of the vegetarian diet (Ophir *et al.*, 1983). However, many of the studies that show differences in blood pressure also show differences in body weight, which is well-known to be related to blood pressure.

Factors other than diet, such as social support within the groups; their non-smoking status; physically active lives and their relatively low body weights, which decrease risks of hypertension and NIDDM, associated with healthy blood lipid profiles, may be involved in lowering the risk of coronary disease in vegetarians.

Neoplasms - Breast Cancer Differences between vegetarians and non-vegetarians in breast cancer incidence and mortality are inconsistent, although cross country comparisons indicate that breast cancer rates are lower in countries that consume vegetarian diets. The best correlations with breast cancer are with total and saturated fatty acid consumption; the consumption of animal fat does not correlate with cancer risk (Carroll and Khor, 1975; Gray *et al.*, 1979). Meat consumption emerged as a strong correlate with breast cancer in the studies by Hirayama (1978), Nomura *et al.* (1978), Lubin *et al.* (1981) and Miller (1978). Seventh-Day Adventist (SDA) women, most of whom are vegetarians, have lower rates of breast cancer than do non-vegetarians, even after the age of menarche is taken into account (Phillips, 1975). However, breast cancer

mortality among Seventh-Day Adventists vegetarian and non-vegetarian women is similar (Phillips and Snowden, 1983). Kinlen *et al.* (1983) found no evidence of a reduction in the proportionate mortality from all cancers and showed an excess mortality from breast cancer and stomach cancer.

Sex hormone levels and the excretion of their metabolites are believed to be associated with breast cancer risk (Moore *et al.*, 1982). Vegetarian women consuming less total or saturated fat, have been shown to have lower circulating sex hormones and higher excretion of oestrogen metabolites (Goldin *et al.*, 1982; Schultz and Leklem, 1983) compared to non-vegetarians. Decreased risk of breast cancer among vegetarians could be attributed to their leanness, lesser use of exogenous oestrogens, lower fat and alcohol intakes and higher intakes of fibre and β -carotene (Dwyer, 1988).

Neoplasms - Large Bowel Cancer In the Western countries cancers of the large bowel are up to ten times those of many Far Eastern and developing nations and it has been suggested that 90% of the variation in rates among countries may be due to diet (Doll and Peto, 1981). Dietary fat, particularly from animal sources (Willet *et al.*, 1990) is known to increase the risk of colon cancer and the higher intake of fibre may be protective against colon cancer and indeed other diseases of the large bowel (Burkitt, 1975). A lower incidence of gallstones (Pixley *et al.*, 1985) and asymptomatic diverticular disease (Gear *et al.*, 1979) have been reported in British vegetarians compared with the general population. The proportion of deaths from colorectal cancer was slightly lower in UK vegetarians compared to the general population (Kinlen *et al.*, 1983). Bingham *et al.* (1985) found that regional large bowel cancer mortality in the UK did not correlate with fibre, fat or beef intake but was negatively associated with high intakes of vegetables high in dietary fibre derived from cellulose and uronic acid. Fibre from fruits or vegetables, but not from cereals, has been consistently associated with a lower risk of colon cancer (Willet, 1989). SDA vegetarians in the U.S.A. have been reported to have significantly lower incidence of colon cancer compared to non-vegetarian Adventists (Phillips *et al.*, 1983). The faecal output of bile acids and neutral sterols is lower in vegans than in non-vegetarians, with lacto-vegetarians being intermediate (Aries, 1971), and a similar trend has been observed in groups of Seventh-Day Adventists of different dietary habits (Turjiman *et al.*, 1984). High fibre intake leading to faster faecal transit time results in decreased degradation of primary bile acids to secondary bile acids

by the intestinal flora. Certain secondary bile acids have been reported to promote colonic tumour growth (Thompson, 1985). Protection against colon cancer in vegetarians may be attributed to increased faecal bulk, lesser colonic cell proliferation and higher serum β -carotene levels (Dwyer, 1988). However, the association between vegetarianism and colon cancer is still unclear, as other religious groups such as Mormons, irrespective of their dietary habits, have lower incidence of colon cancer compared to the general population (Enstrom, 1978).

1:10 Caucasian Vegetarians versus Indian Vegetarians

Indians are vegetarians essentially by tradition and by virtue of being Hindus, unlike the Caucasians who adopt vegetarianism for a variety of reasons, primarily because it is healthy. The change from an omnivorous diet to a vegetarian diet in the Caucasians is often accompanied by a conscious change in lifestyle such as: high awareness of health issues, taking more exercise, abstaining from smoking and, importantly, a conscious effort to achieve a balanced diet by including a variety of foods. Differences in types of food consumed by Asians and non-Asian vegetarians have been observed, notably with respect to milk and butter which were more popular with Asians, while margarine consumption was lower (Bull and Barber, 1984). However, this was based on household food consumption data which does not accurately reflect individual dietary intakes. Nutrient intakes in Asian women have been reported in pregnancy only, while information on the intakes of Caucasian vegetarian women is limited.

Studies conducted on Caucasian vegetarians generally conclude that vegetarianism does offer a certain protection against coronary heart disease, diabetes and intestinal cancers, without considering the variability of diets amongst different groups of vegetarians. Asians living in Britain, despite being predominantly vegetarian, appear to show higher incidence of coronary heart disease and diabetes. However, they seem to have low susceptibility to cancers of all sites, particularly breast and intestinal, except for liver cancer which may be a consequence of past viral infection. Both Indian and Caucasian vegetarian women seem to be susceptible to nutritional anaemias but Indian women also suffer from osteoporosis and vitamin D deficiency.

Therefore, there is a need to characterize vegetarian diets of different groups in order to assess their influence on health and disease. Vegetarian diets differ specifically with regard to their fat and fibre content which may be involved in the aetiology of cardio-vascular diseases and certain types of cancer.

INTRODUCTION

PART II

1:11 Dietary Fat and Coronary Heart Disease

1:11:1 Pathology of Coronary Heart Disease

Coronary heart disease (CHD) is a major public health problem affecting a high proportion of middle-aged men. Women, at least before menopause, are less prone to the disease than men. CHD and ischaemic heart disease are synonymous names for a group of syndromes arising from the failure of the coronary arteries to supply sufficient blood to the myocardium and are often associated with atherosclerosis of the coronary arteries. CHD usually manifests in three important disorders; myocardial infarction, angina pectoris and sudden cardiac death.

Myocardial infarction is the necrosis or destruction of part of the heart muscle due to the failure of blood supply. It may lead to sudden death, or heal leaving a lesion which may restrict physical activity of the patient. However, some may return to their normal life and activities but they are at increased risk of re-infarction. Infarction is usually due to thrombus formation in atherosclerotic coronary artery which obstructs the lumen, thus preventing normal blood supply to the cardiac muscle. In the absence of thrombus, infarction could also arise because the lumen of the coronary artery is so narrowed by atherosclerosis that the blood flow is insufficient to supply the oxygen required to maintain the cardiac muscle.

Angina pectoris is characterised by severe chest pain provoked by physical exercise or excitement, thereby limiting the patient's physical activities, but they may be free from disability as long as they keep within the limits of their exercise tolerance. However, they also carry an increased risk of myocardial infarction or sudden death on unusual exertion. Although sudden death may occur in known angina pectoris or myocardial infarction, it can also be due to cardiac arrest or ventricular fibrillation resulting from minor or painless ischaemia sufficient to interrupt the electrical conduction system of the heart. Whatever the outcome, the main underlying feature in these disorders is atherosclerosis and insufficient oxygenation of cardiac muscle.

The atherosclerotic lesion in man is characterized by accumulation of lipid in and around cells of the intimal space of the arteries and is associated with a cellular and fibrous proliferation which leads to a narrowing of the lumen of the vessel. Ross *et al.* (1984) propose that lesions occur in response to injury or mechanical trauma on the

intimal surface resulting in platelet aggregation and formation of thrombus which sticks to the vessel wall. Platelets, leucocytes and tissue macrophages thus attracted to the site of injury also induce smooth muscle cells to proliferate and produce a raised gelatinous lesion. The lesion either heals (visible as a fatty streak in young children) or goes on to accumulate lipid forming a complex atheroma consisting of fibrous tissue and lipid which may eventually be enclosed by endothelium. Hence, it could be assumed that an increased tendency to thrombosis or inefficient fibrinolytic mechanism may be responsible for atherosclerosis. *Post mortem* coronary angiographic studies show that coronary occlusion is most commonly caused by a blood clot resulting from the rupture of an atherosclerotic plaque. Therefore, the pathology of CHD implies that several factors interact to produce the disease: the generation of the lesion, haemostatic variable such as blood pressure, blood viscosity, cellular components and the coagulation and fibrinolytic pathways.

Dietary fat is known to influence these factors, particularly thrombotic tendency and atherogenesis. The lipid deposited in atheroma consists of cholesterol derived from plasma lipoproteins which, in turn, are influenced by the quality of dietary fat.

1:11:2 Epidemiological Associations of CHD with Fat Intake

Interpopulation Comparisons

Although CHD is a multifactorial disease with smoking and hypertension being the major risk factors, diet appears to play a central role as CHD mortality rates are the highest in North America, northern Europe and Australasia, where most of the dietary fat is derived from meat and dairy products (Thom *et al.*, 1985). In contrast, CHD mortality rate is low in southern Mediterranean countries, where olive oil is the main source of dietary fat. Despite the higher mortality from other types of cardio-vascular disease such as stroke, mortality from CHD remains low in Japan and Greenland Eskimos. A decline in CHD mortality has been observed in several countries over the past 20 years, notably in the United States, Finland and Australia. This may be a consequence of change in the quality of fat consumed (saturated to polyunsaturated fatty acids) because there has been little change in the total quantity of dietary fat.

Transmigration studies suggest that populations migrating from an area of low incidence of CHD to one of high incidence acquire the disease pattern of the new

country, as observed in the Japanese living in Hawaii and California, who have mortality rates from CHD similar to the Americans. Migration in the opposite direction to areas where incidence is low has been accompanied by a low risk of CHD: the incidence of CHD in second generation Jewish immigrants to Israel is lower than in New York. However, CHD rates in Asian men of Indian descent living in the U.K. is higher than in the indigenous population. It is uncertain whether this susceptibility is acquired in the host country or is innate in this group. Incidence of CHD is high in urban India, almost comparable to the rates observed in Indian migrants in other parts of the world.

International comparisons show an association between fat consumption and CHD which is more evident when fat is expressed as a percentage of the energy intake. CHD has been called a 'disease of affluence' and consumption of fat is known to increase with affluence. However, there are populations with a high proportion of energy derived from fat and a low incidence of CHD; for example the Greenland Eskimos (Bang and Dyerberg, 1980) and South Pacific Islanders (Prior and Evans, 1970). Keys (1970) in the Seven Countries Study, showed that the national percentage of energy derived from saturated fat was correlated with death from CHD and with serum cholesterol. An extremely low incidence of CHD and atherosclerosis has also been observed in populations that eat large amounts of C20-C22 polyunsaturated fatty acids derived from fish or marine mammals.

Intrapopulation Studies

In contrast to the between-country association, the relationship between saturated fat intake and risk of CHD within the general population of any particular country is either non-existent or very weak (McGill, 1979). The diet in the Western countries is relatively homogenous with respect to its fatty acid composition, but difficulties are encountered in accurate assessment of individual fatty acid intakes which may explain the lack of clear association between saturated fat intake and risk of CHD. However, comparisons made between groups with widely differing saturated fat intakes do show a relationship between saturated fat intake and CHD. Vegans who consume no animal fats and whose diets contain 10% of energy from saturated fat have a lower incidence of CHD than lactovegetarians and omnivores (Phillips *et al.*, 1978).

The level of linoleic acid in adipose tissue is believed to be a good indicator of polyunsaturated fat intake. Scottish men were reported to have lower linoleic acid levels in adipose tissue compared to Swedish men (Logan *et al.*, 1984), and later studies in the Scottish populations revealed lower levels of linoleic acid in patients with angina than in unaffected controls (Wood *et al.*, 1987). However, adipose tissue linoleic acid levels in smokers have been observed to be lower than in non-smokers and the association between linoleic acid and heart disease may be obscured by smoking habits of the population, which in itself is a major risk factor of CHD. Also, Asian men of Indian descent in the U.K. have higher concentrations of linoleic acid in plasma phospholipids but are at greater risk of CHD compared to the indigenous population (Reddy and Sanders, 1987). Intakes of polyunsaturated fatty acids (EPA and DHA) of marine origin have been reported as being negatively associated with risk of CHD and, recently, substituting meat with oily fish in the diets of survivors of myocardial infarction has shown a considerable reduction in the rate of reinfarction in these patients (Burr *et al.*, 1989).

1:11:3 Plasma Lipoproteins

Lipids, being insoluble in water, circulate in plasma in association with certain specific proteins called apolipoproteins. Plasma lipoproteins are lipid — protein complexes of density < 1.21 g/ml present in the plasma. They are classified in terms of their hydrated density and determined in the preparative ultracentrifuge as they are lighter than other plasma proteins because of their high and variable lipid content. There are four classes of lipoproteins in fasting plasma very low density lipoprotein (VLDL, density < 1.006 g/ml); intermediate density lipoprotein (IDL, density 1.006-1.019 g/ml); low density lipoprotein (LDL, density 1.019-1.063 g/ml) and high density lipoprotein (HDL, density 1.063-1.21 g/ml). Chylomicrons appear in postprandial plasma a few hours after a fatty meal and are cleared from the circulation.

The primary function of plasma lipoproteins is lipid transport, mainly triglycerides and cholesterol, to specific cells. Cholesterol is not used for energy but is a precursor of steroid hormones and bile acids and is a structural component of cellular membranes. It is transported as cholesteryl esters by the lipoproteins. VLDL and chylomicrons are composed primarily of triglycerides. Chylomicrons transport exogenous (dietary) triglyceride and VLDLs transport endogenous triglyceride. VLDL normally contains 10-15% of the total plasma cholesterol while LDL contains 60-70%. HDLs are

approximately half protein and half lipid and usually contain 20-30% of the total plasma cholesterol.

VLDL is secreted mainly by the liver and is responsible for the endogenous transport of triglycerides to peripheral tissues. The hepatic VLDL particles contain apolipoprotein B100 (apoB-100) and a triglyceride-rich core. As soon as nascent VLDL enters the plasma it acquires apolipoprotein C (apoC) and cholesterol ester, probably from HDL. In the capillaries of adipose tissue and muscle the VLDL triglycerides are hydrolysed by endothelial lipoprotein lipase which is activated by apoC, resulting in 80-90% loss of triglyceride and the loss of apoC. The product of this conversion, IDL, is relatively rich in cholesteryl esters and contains apoB-100 and apolipoprotein E (apoE). IDL particles containing apoE are rapidly cleared from the plasma by hepatic uptake facilitated by receptors to apoE. IDL that escapes hepatic uptake is further delipidated and loses its apoE to form LDL (Grundy, 1984). The LDL particle is enriched in cholesteryl ester and contains apoB-100. It appears that only smaller VLDL particles skip hepatic uptake to form LDL (Packard *et al.*, 1984).

The concentration of LDL in plasma is not only dependent on its rate of synthesis but also on its rate of removal. Two-thirds of the LDL particles are metabolised after binding to a specific receptor for apoB-100 located on the surface of hepatic and other body cells, leading to cellular uptake and degradation of LDL. This receptor-mediated uptake helps to meet the cholesterol needs of the cell and suppresses its synthesis within the cell by inhibiting HMG CoA-reductase, a rate-limiting enzyme involved in the synthesis of cholesterol. In normal human subjects the remaining LDL that is not taken up by the receptors is metabolised by alternative mechanisms involving modification of LDL particles by glycolysation or oxidation. Modified LDL is taken up by macrophages and can lead to foam cell formation and atherosclerosis (Figure 1:2).

The transport of exogenous lipid differs from the endogenous pathway. Dietary fatty acids (>C12), together with dietary cholesterol, are incorporated as triglycerides into chylomicrons in the small intestine. Chylomicrons are similar to VLDL except that they contain apoB-48 instead of apoB-100 and acquire apoB and apoC from HDL in circulation. Triglycerides in chylomicrons are rapidly hydrolysed by endothelial lipoprotein lipase leaving a chylomicron remnant which is rapidly cleared by hepatic

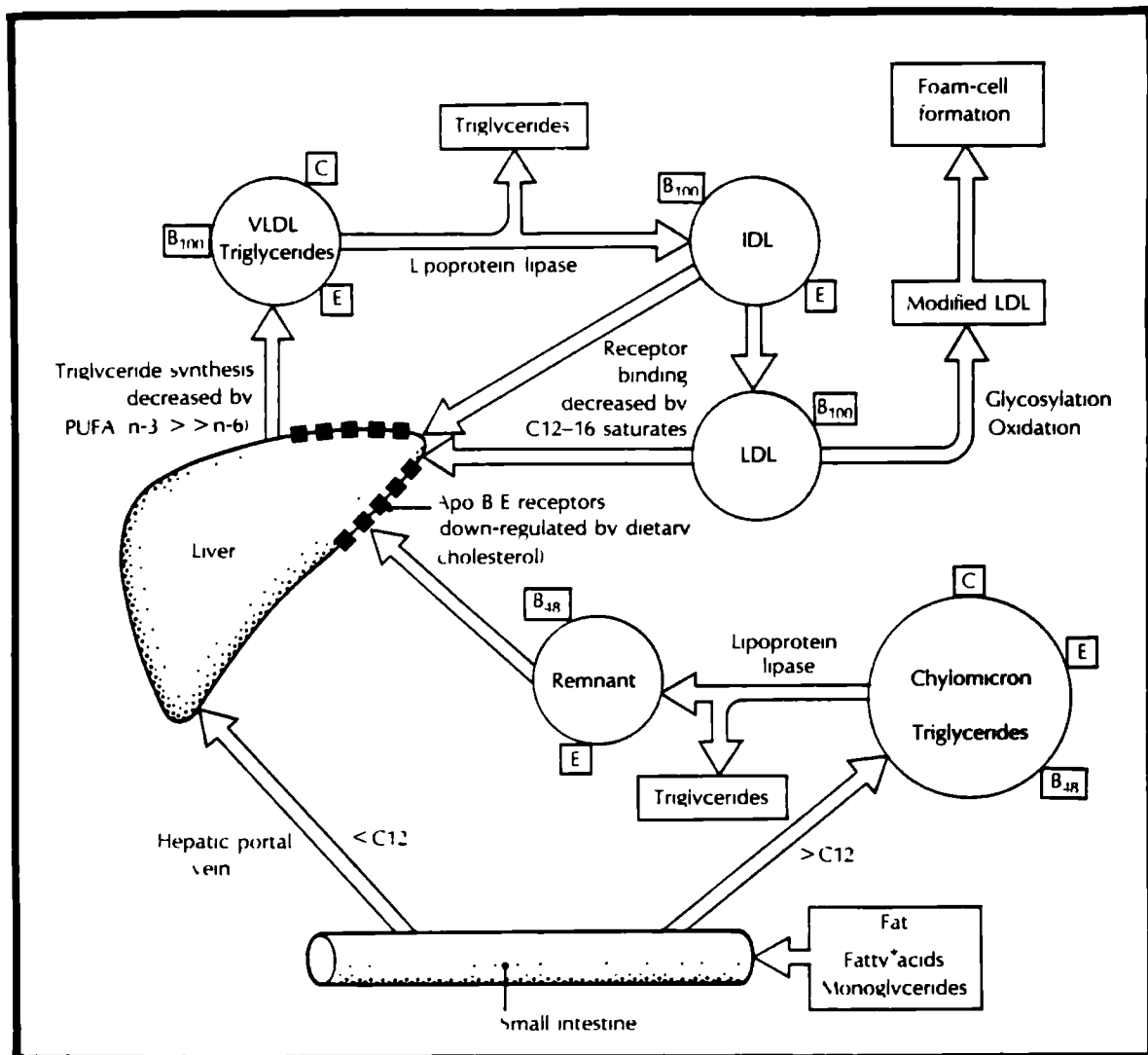


Figure 1:2 Influence of dietary fat on lipoprotein metabolism

uptake. Chylomicron remnants are themselves not converted to LDL but a high intake of dietary fat can compromise the endogenous lipoprotein pathway by competitively inhibiting the clearance and uptake of lipoproteins.

HDL Cholesterol

HDL is heterogenous in nature with sub-classes (HDL₂ and HDL₃) varying in their protein and lipid content, the percentage of protein varying from 60-40% in the least dense particles. The main proteins associated with HDL are apoAI and apoAII with the ratio of AI/AII tending to be higher in HDL₂. ApoC is also present in HDL to variable extents depending on the flux of the triglyceride-rich lipoproteins. In humans, small quantities of HDL₁ containing apoE have been identified with density lying between that of LDL and HDL₂. This may confer HDL₁ an affinity for LDL receptors and distinguishes it metabolically from the bulk of the HDL which lacks apoE. The lipid component of HDL sub-classes also varies, particularly that of free cholesterol and phospholipid increasing with decreasing density of the particles.

The concentrations of HDL and its components in serum result from the interplay of a large number of intravascular and cellular metabolic events (Nicoll *et al.*, 1980). The bulk of HDL appears to arise from the interaction of nascent HDL₁ secreted by the liver and intestine, with lipids and proteins released during the catabolism of triglyceride-rich lipoproteins. A portion of HDL lipid also arises from transfer and uptake of lipids, particularly free cholesterol from cell membranes. Several enzyme activities are known to determine HDL lipid concentrations, notably lipoprotein lipase in the capillary endothelium of extra-hepatic tissues, which is rate-limiting in the hydrolysis of lipoprotein triglycerides (Krauss, 1982). *In vitro* and *in vivo* studies have shown that during the course of hydrolysis of triglyceride-rich lipoproteins by lipoprotein lipase, surface lipids from these particles can be transferred to HDL₃, resulting in the formation of HDL₂-like particles. Fractional catabolic rate of plasma triglycerides has also been found to correlate with levels of HDL cholesterol in normolipemic subjects (Kekki, 1980). Human HDL lipids have also been reported to be substrates for hepatic lipase activity which is known to be reduced in conditions associated with increased levels of HDL₂ (Kuusi *et al.*, 1980). An enzyme of major importance in HDL metabolism is lecithin : cholesterol acyltransferase (LCAT) which is activated by apoAI and is responsible for esterification of cholesterol in HDL, particularly HDL₃.

In human plasma, cholesteryl esters are generated in HDL by the LCAT reaction and are then transferred to VLDL and LDL by a cholesteryl-ester transfer protein (Myant, 1990). Therefore, HDL plays a vital role in the clearance of lipids and metabolism of VLDL and LDL through the mediation of its apoproteins. It also acts as a scavenger of free cholesterol from extra-hepatic tissues, transporting it to the liver to be excreted as bile acids, 'reverse cholesterol transport', thus aiding in regulating cholesterol levels in the body.

Factors Affecting HDL Concentrations

One of the major constitutional factors to affect HDL levels is gender. In most populations (Maoris being one exception) it has been demonstrated that women have higher levels of HDL than men at all ages following puberty, but HDL₃ levels are actually lower in women than men (Anderson *et al.*, 1978). ApoAI has also been reported to be higher in women (Cheung and Albers, 1977). Hormonal influences are known to alter HDL levels, exogenous androgen administration and onset of puberty have been reported to decrease HDL concentrations in men (Krauss, 1982). Fluctuations in HDL concentration observed during the normal menstrual cycle may be due to hormonal effects because transient increases in HDL₂ have been reported to occur at or near the time of ovulation (Krauss *et al.*, 1979). Evidence for autosomal dominant inheritance of low HDL levels has been reported in a large kindred with a high prevalence of coronary disease (Verganic and Bettale, 1981).

Ethnic differences in HDL levels have been observed in comparisons of various population groups: for example Eskimos in Greenland and Denmark compared to Caucasians in Denmark; Maoris and non-Maoris in New Zealand and black and white Americans in the U.S.A. An age effect has been reported, with HDL levels decreasing with age in men and increasing in women up to the age of 60 year, after which no age effect is apparent (Heiss *et al.*, 1980).

Obesity has been repeatedly shown to be inversely associated with HDL cholesterol in both sexes. Data from the LRC Prevalence Study suggest that persons in the highest decile of body mass have HDL concentrations approximately 10 to 15% lower than those in the lowest decile (Glueck *et al.*, 1980). In the same study, a significant negative correlation between obesity and HDL was found in both men and women,

independent of age, smoking, alcohol and oestrogen use. Centripetal body fat patterning in women is also associated with decreases in HDL cholesterol, regardless of total body fat (Baumgartner *et al.*, 1987). Weight loss has also been associated with an increase in HDL. Physical exercise is believed to increase HDL. Wood *et al.* (1977) reported 34% higher HDL levels in women runners compared to non-runners and a similar observation was made in male runners.

Several epidemiological studies have consistently shown a positive association between alcohol consumption and HDL concentrations. Frimpong and Lapp (1989) demonstrated a significant increase in HDL-C during alcohol consumption and a decrease during abstention to initial values in both groups of variable and fixed drinkers. It has been reported that alcohol consumption is only marginally related to HDL₂ in women, but significantly so in men, whereas consumption is significantly associated with HDL₃ in women, but only minimally so in men (Haffner *et al.*, 1986). However, Gregory *et al.* (1990) showed that alcohol intake was a significant predictor of HDL cholesterol in both men and women.

Lipoprotein(a) or Lp(a)

Lp(a) is essentially an LDL particle in which apoB-100, the protein moiety of LDL, is linked to a unique apoprotein, apo(a), by one or two disulphide bridges. Apo(a), which is a specific marker of Lp(a), is a highly glycosylated protein (28% carbohydrate by weight) displaying heterogeneity in size which may be related both to polypeptide chain polymorphism and to variation in extent of glycosylation (Loscalzo, 1990). Apo(a) has a striking structural similarity to plasminogen, but cannot be activated to plasmin, an enzyme involved in the fibrinolytic system. Lp(a) concentrations are genetically determined with a wide concentration distribution and a positive skew (geometric mean 10 mg/dl). Seventy percent of the variation is believed to be genetic (The Lancet, 1991).

Utterman *et al.* (1988) showed an inverse relationship between apo(a) size and plasma concentration and suggest that a genetic variation in the apo(a) gene locus controls apo(a) size and Lp(a) concentrations in plasma. The primary site of synthesis of Lp(a) appears to be the hepatocyte and, like apoB-100, apo(a) is also synthesized in the liver. The concentration of apo(a) in serum is believed to be an indicator of Lp(a) levels in plasma and an upper normal level of 7 mg/dl has been suggested.

High plasma levels (> 30 mg/dl) of Lp(a) have been associated with an increased incidence of atherosclerotic vascular disease (Scanu and Fless, 1990). On the basis of their structural make-up, Lp(a) particles may be either pro-atherogenic, pro-thrombogenic, or both. It is plausible that Lp(a) particles may be taken up by the scavenger pathway. In situations where Lp(a) levels are elevated, it is possible that Lp(a) particles transverse the endothelium of the arterial wall and become entrapped in the intima. Lp(a) has been found in the arterial wall and may participate in the formation of atherosclerotic plaque (Scanu, 1990). A number of studies *in vitro* and *ex vivo* have shown that Lp(a) can compete with plasminogen for binding to the plasminogen receptor and thus result in accumulation of plasminogen, creating a thrombogenic state. An imbalance between plasma levels of Lp(a) and plasminogen would occur only when Lp(a) levels are markedly high, but no relationship between Lp(a) concentration and fibrinolytic activity has ever been demonstrated *in vivo*.

A co-localization of Lp(a) and fibrin has been shown to take place at the level of arterial wall, and thus complexation may lead to modification of Lp(a) surface and preferential uptake by the scavenger receptor pathway. The atherogenic and thrombogenic potential of Lp(a) is apparent. However, the degree of pathogenicity is likely to be dependent on genetic predisposition of any given individual on the presence of other risk factors such as cigarette smoking, hypertension, diabetes and obesity. Diet, however, seems to exert little influence on plasma levels of Lp(a).

1:11:4 Dietary Fat: Effect on Lipid Metabolism

The epidemiological associations between high intake of saturated fatty acids, serum cholesterol concentrations and CHD are well known. It is essential to discriminate between cholesterol carried by LDL and that transported in the form of HDL, because high concentrations of LDL which carry 60-70% of total cholesterol in plasma are implicated in atherogenesis. High levels of HDL, on the other hand, are protective against atherosclerosis. Any dietary change in order to reduce the risk of CHD should aim at lowering total cholesterol and LDL-cholesterol concentrations. Elevated levels of VLDL and IDL are associated with increased risk of CHD and raised plasma triglyceride levels are usually found in association with elevated VLDL and IDL.

Effect of Dietary Cholesterol

Cholesterol in the diet has a limited effect on plasma cholesterol concentrations. The first 200 mg appears to be most effective in raising plasma concentrations (Keys, 1984) and for intakes above that each 100 mg of dietary cholesterol could lead to an increase in plasma cholesterol of about 4 mg/dl (Hegsted, 1986). The National Diet-Heart Study (1968) indicated that the dietary cholesterol-induced increase in serum cholesterol was somewhat smaller on a diet high in polyunsaturated fatty acids than on a diet high in saturated fatty acids. Therefore, there seems to be an interaction of dietary fat type with dietary cholesterol and it is notable that foods high in saturated fat also contribute substantially to dietary cholesterol. High dietary intakes of cholesterol are believed to decrease the number of LDL-receptors in the liver and thus reduce the fractional catabolic acid of LDL (Packard *et al.*, 1983) contributing to an increase in total cholesterol levels in plasma.

It is argued that there may be genetic variability in dietary responses to cholesterol and saturated fat. Patients with Apo E4:E4 phenotype seem to be more responsive than those with Apo E3:E3 (Miettinen *et al.*, 1988). Recently Kern (1991) reported the case of an 88 year old man who ate 25 eggs per day, contributing 12,953 μ mol of dietary cholesterol. His plasma cholesterol level was 200 mg/dl (5.18 mmol/l) and he was also free from clinically important atherosclerosis. It is suggested that in some individuals there may be adaptation to high intake of dietary cholesterol through decreased absorption and an increased conversion to and excretion of bile acids (Gotto, 1991).

Effects of Saturated Fat

Fatty acids with a chain length of C4 to C10 which make up about 12% of the total saturated fatty acids in coconut, palm kernel oil and butter fat, appear to have no effect on serum total cholesterol relative to carbohydrates or oleic acid (Beynen and Katan, 1989). However, there is controversy about the relative effects of lauric (C12) and myristic (C14) which were shown to be more hypercholesterolaemic than palmitic acid (Vergroesen and De Boer, 1971). McGandy *et al.* (1970) observed that myristic acid and palmitic acid were equally active in elevating serum cholesterol, their effect being greater than that of lauric acid. Stearic acid (C18), like short chain fatty acids, has been shown to have no effect on serum cholesterol (Hegsted *et al.*, 1965; Bonanome and Grundy, 1988), probably because it is rapidly converted into oleic acid in the body.

Lipoprotein studies in man have found that the synthetic rate of LDL is increased with a high saturated fat diet compared with a high polyunsaturated fat diet. It is known that all VLDL is not converted to LDL and it seems that only small particles of VLDL are converted into LDL, while the large particles are removed rapidly from circulation without being converted into LDL. Factors such as high intake of carbohydrate that increases VLDL triglycerides and VLDL size often do not lead to an increase in LDL (Sanders, 1990). It is generally agreed that energy from saturated fat more than 10% of total energy intakes are not desirable (European Atherosclerosis Society, 1987).

Effect of Monounsaturated Fatty Acids

Oleic acid (*cis*-isomer C18:1) is the most abundant monounsaturated fatty acid in the diet and is found in various plant oils ranging from 65-85% of the total fat in olive oil, rapeseed oil, etc. Dietary studies on plasma cholesterol levels show that monounsaturated fatty acids are neutral with regard to their cholesterolaemic effects. Replacement of saturated fat with linoleic or oleic acid has been reported to have an equivalent LDL cholesterol lowering effect (Mattson and Grundy, 1986; Mensink and Katan, 1989). Studies on effects of elaidic acid (*trans*-isomer C18:1) usually found in hydrogenated fats such as margarines, have shown a similar neutral effect on plasma cholesterol as oleic acid in man (Mattson *et al.*, 1975). As olive oil has a long history of use and is associated with a low incidence of CHD, especially in Mediterranean countries, it is regarded as a safe alternative to saturated fat.

Effects of Polyunsaturated Fatty Acids

n-6 Fatty Acids Linoleic acid is the most abundant of the *n-6* series of fatty acids. Polyunsaturated oils such as sunflower and corn oils have traditionally been advocated for the replacement of saturated fat. For each 1% of energy from saturated fat replaced by linoleic acid there is a 5 mg/dl reduction in plasma cholesterol. Intakes of linoleic acid of up to 12% of energy do not lower HDL (Mensink and Katan, 1989) but higher intakes do (Mattson and Grundy, 1986). Increasing the proportion of linoleic acid from 4% to 10% of energy intake has little additional benefit (Kuusi *et al.*, 1985) in terms of cholesterol reduction if saturated fat intakes are kept constant. It is well known that plasma LDL cholesterol levels can be lowered by changing the P/S ratio of the diet. This change in LDL is determined by the rate of secretion of VLDL, its conversion to LDL and the rate of removal of LDL regulated by apoB.

Human studies show a marked reduction in LDL synthesis rate when linoleic acid replaces saturated fatty acids in the diet (Illingworth *et al.*, 1981; Cortese *et al.*, 1983) and a slight rise in fractional catabolic rate. An increase in LDL receptor activity reported in animal studies (Spady and Dietschy, 1989) however, may not be due to an increase in the number of receptors (Sorci-Thomas *et al.*, 1989). It seems likely that the saturated fatty acids somehow interfere with the binding of LDL to the apoB receptor.

n-3 Fatty Acids There are two types of n-3 fatty acids in the human diet - linolenic acid (C18:3 n-3) found in soyabean and rapeseed oils (10%) and in linseed oil (50%); marine oils contain the very long chain eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6). The n-3 polyunsaturated fatty acids have different effects on plasma lipids from linoleic acid and the effects observed are strongly dose-related. Both EPA and DHA can markedly lower plasma triglycerides and VLDL concentrations (Harris, 1989; Sanders *et al.*, 1989), while linoleic acid and linolenic acid do not show the same effect at comparable doses (McDonald *et al.*, 1989).

Studies in healthy volunteers do not show any significant change in total or LDL cholesterol with moderate intakes of fish oil but very high intakes of fish oil (24 g of C20-C22 n-3/day) do lower the concentration of both LDL cholesterol and LDL apoprotein B by decreasing the rate of LDL synthesis (Harris, 1989). The smaller LDL pool size may be due to reduced synthesis rather than any increase in its fractional catabolic rate, which might imply down-regulation of the LDL receptors by fish oils. Dietary EPA and DHA decrease LCAT activity which may be accompanied by an increase in the HDL₂:HDL₃ ratio (Abbey *et al.*, 1990), thereby increasing HDL₂ cholesterol. Chylomicron clearance is increased in subjects following the consumption of oily fish (Harris, 1989) and fish oil also tends to lead to less postprandial hyperlipaemia compared to olive oil or saturated fat (Weintraub *et al.*, 1988; Sanders *et al.*, 1989).

A study by Hermann *et al.* (1989) reported that fish oil supplementation led to a significant reduction of Lp(a) in patients with elevated levels, but this needs further confirmation. The consumption of n-3 fatty acids resulting in altered fatty acid composition of lipoproteins may in itself be important to their anti-atherogenic potential.

1:11:5 Atherosclerosis in Animals

The relationship of dietary fatty acid saturation to experimental atherosclerosis in animals parallels closely the fatty acid effects on levels of serum cholesterol, particularly LDL-cholesterol. It is possible to induce a form of atherosclerosis in animals by feeding large amounts of cholesterol in the diet, usually 1-2% by weight, an amount 50-100 times greater than in human diets. The lesions seen in these animals are unlike those seen in humans. Human hypercholesterolaemia is characterized by increased concentrations of LDL, whereas induced hypercholesterolaemia in experimental animals is associated with increased VLDL rich in cholesteryl esters. An increase in VLDL may be due to increased hepatic synthesis in response to dietary cholesterol or may represent remnants of intestinal lipoproteins.

Elevation of VLDL will cause macrophages to accumulate cholesteryl esters and resemble foam cells in the external wall (Sanders, 1990). Rabbits, pigs and certain primates, when fed diets containing a high proportion of saturated fat over a long period, develop lesions that are more similar to those in humans (Willser and Visselinovich, 1988). The severity of experimental atherosclerosis is influenced by the type rather than the quantity of fat in the diet. When unsaturated vegetable oils such as sunflower oil are substituted for butter fat, the resulting atherosclerosis was less severe (Mendelsohn and Mendelsohn, 1989). Generally, saturated fats such as butter, tallow lard and hardened coconut oil have been found to be atherogenic, but palm oil and cocoa butter appear to be exceptions. Monounsaturated fatty acids such as oleic acid do not appear to be atherogenic in animals. Polyunsaturated fatty acids from fish oils inhibit atherogenesis in pigs, dogs and monkeys, even in the presence of hypercholesterolaemia (Weiner *et al.*, 1986) but not in rabbits.

1:11:6 Trials of Diet in the Prevention of Coronary Heart Disease

A number of trials have been carried out to test whether changing the nature of dietary fat intake in mid-life reduces the risk of death from CHD. Details of randomized trials conducted are summarised in Table 1:5

A majority of trials were multifactorial in nature and the effects of change in plasma lipids were confounded with effects of change in cigarette smoking and blood pressure, making it difficult to interpret the results. Multiple risk factor intervention

TABLE 1:5 Summary of randomized controlled trials on diet and prevention of CHD

Study	Study Population	Serum Cholesterol at Entry	Interventions and Targets of Intervention	% Differences between Treated and Control Groups	
				Serum Cholesterol	CHD
Göteborg Multifactor Trial (Wilhelmsen <i>et al.</i> , 1986)	20,015 men aged 47 to 55	250	Diet, smoking, high blood pressure	0	0**
WHO Multifactor Trial (WHO Collaborative Group, 1983)	49,781 men aged 40 to 59	216	Diet, smoking, high blood pressure	-1	-7*
Multiple Risk Factor Intervention Trial (MRFIT, 1982)	12,886 high risk men aged 35 to 57	254	Diet, smoking, high blood pressure	-2	-7*
Los Angeles Veterans Administration Domiciliary Study (Dayton <i>et al.</i> , 1968)	846 men aged 55 to 89 20.1% had evidence of MI	233	Diet only	-13	-24**
Oslo Study (Hjermann <i>et al.</i> , 1981)	1,232 normotensive men aged 40 to 49	329	Diet and smoking	-13	-47** ($p < 0.05$)
Finnish Mental Hospital Study (Meitinen <i>et al.</i> , 1972)	4,178 men, 6,434 female patients aged 15+	267	Diet only	-15	-53* ($p < 0.05$)

* CHD death was the end point

** CHD death and non-fatal myocardial infarction (MI) were end points

trials have shown little benefit in terms of increased life expectancy (McCormick and Skrabanek, 1988). It is generally believed that dietary intervention in mid-life has little impact on CHD mortality rates, but two trials have shown benefits from changes in fat intake in younger men. Subjects in the Oslo study were urged to give up smoking and to reduce total fat and saturated fat intake, which was achieved by decreasing the consumption of dairy fat and changing from fatty meat to fish and poultry. Total fat intake was reduced from 44% to 28% of the dietary energy and saturated fatty acid intakes were reduced from 18% to 8%. The risk of CHD was significantly reduced, which was greater than that predicted from giving up smoking. Total mortality was not significantly lower in the first analyses but the 8-year follow-up data showed a significant decrease in all cause mortality (Hjermann *et al.*, 1986). The Finnish Mental Hospital trial with a cross-over design, where saturated fat was replaced with polyunsaturated fats, resulted in a reduction of death rate from CHD, but total mortality was unaffected.

Burr *et al.* (1989) carried out a secondary intervention trial in 2,000 patients of non-fatal myocardial infarction. A factorial design was used and patients were advised to decrease total fat, especially saturated fat, or to increase oily fish consumption, or to increase cereal fibre intake. No significant changes occurred in CHD rate or total mortality in the groups who received fat or fibre advice, but the group given the fish advice showed a 29% reduction in total mortality over the 2-year period, which was mainly a result of a reduction in fatal myocardial infarction. The total CHD incidence was, however, lower but not significantly so. From the above table it is evident that trials which achieved less reduction in total cholesterol made little difference to the rate of CHD, whereas those that achieved greater reduction in serum cholesterol also resulted in a considerable decrease in CHD.

1:12 Dietary Fat and Cancer

Cancer is a malignant disorder of cell proliferation, fundamental to this being loss of control of cell growth and the tissue proliferates independently of 'normal' growth signals of the body. Cancer may originate from a single altered cell, with considerable variations occurring during the proliferation stages, making it difficult for cancers even of the same site to be viewed in a generalized way (Taussig, 1987). The development of cancer is thought to involve two stages: initiation and promotion. Initiation is regarded as being mutagenic, mutagen being chemical or a physical agent that interacts

with DNA causing a permanent transmissible change in the genetic material of a cell which is irreversible. For development into a tumour promotion is required which may take many years and is thought to be reversible. This sequence of events has been clearly demonstrated in animal experiments but it is uncertain whether a similar sequence occurs in man. For example, as in the case of cigarette smoke, no distinction has been made for its role either as an initiator or promoter, although its role as a cancer causing agent is well established (Higginson and Muir, 1979).

Various naturally-occurring chemicals in our diets, as well as those formed during food processing, storage and preparation such as nitrates, nitrites, polycyclic aromatic hydrocarbons, are known to be mutagenic or carcinogenic in animal models, acting mainly as initiators. Free radical attack is probably the initial damage that begins the process of malignant transformation (Ames, 1979). Oxygen radicals and/or hydrogen peroxide may be generated by compounds or agents that act as promoters (Marx, 1983; Emerit *et al.*, 1983). Free radicals have the capacity to interact with a wide variety of biological molecules, but because of their double bonds, unsaturated fatty acids are particularly vulnerable. The resulting lipoperoxides react with many cellular compounds, including DNA, forming cross-links in the proteins and thereby damaging cell membranes and DNA (Weinstein *et al.*, 1984). Thus promoters may not interact directly with DNA. Other molecules subject to peroxidation, such as prostaglandins and leukotrienes, derivatives of arachidonic acid which play a major role in cell growth and differentiation (Ohuchi and Levine, 1980). Metaplasia and abnormal changes in cells often occur during tumour development and these changes may be prevented by dietary factors, particularly vitamin A and retinoids, which help to maintain normal cell differentiation (Uriel, 1979). Dietary factors may play a role in inhibiting tumour growth and in strengthening the host's immunological defence (Williams and Dickerson, 1990).

Epidemiological studies that have shown correlations between international food consumption patterns and disease incidence were for cancers of the gastrointestinal tract and endocrine-related tumours such as breast, uterus, ovary and prostate. Strong associations were also found between a high consumption of nitrates and low consumption of vitamin C or fruit and gastric cancers (Miller, 1985). Some reports also suggested that vitamin A or its precursor carotenoids, found in green and yellow vegetables, may be protective against lung cancer (Peto *et al.*, 1981). Consistently positive associations

between alcohol consumption and oesophageal cancer were also reported (Chilvers *et al.*, 1979). The most consistent relationship reported is the strong positive association between the consumption of total fat and death rates from breast and colon cancer (Correa, 1981) and negative associations between dietary fibre and colon cancer (Armstrong and Dolls, 1975). However, reports of associations between the intake of fat and cancers of breast and colon and between intake of fibre and cancer of colon obtained from cross-sectional studies have not been consistently supported by case-control studies (Byers, 1988; Berrino and Muti, 1989). This lack of agreement between cross-cultural and case-control studies has in part been attributed to inaccuracies in the methods available (usually food frequency questionnaires) for assessing intakes of dietary fat and fibre in large numbers of individuals. In contrast, micronutrients such as carotene are derived from few foods in the daily diet and could be quantified reliably by food-frequency questionnaires, which may explain the consistent association found between carotene intake and lung cancer in both cross-cultural as well as case-control studies.

Dietary fat is the component for which there appears to be the most casual association with cancer and it is most closely linked to enhancement of carcinogens (NRC, 1989). The involvement of fat in the development of cancer has been known for a long time (Tannenbaum, 1942). Dietary fat has been shown to enhance tumour formation after a carcinogen has been fed to experimental animals. Thus fat is likely to be involved in the 'promotion' of, rather than the 'initiation' process of carcinogenesis (Carroll and Khor, 1975). The promotional effects of high fat diets increases with increased dose of carcinogen to a limit of 20% fat content of diet, after which no further effect is seen (NRC, 1989). Other studies have shown that prolonging feeding time enhances the effect of diet. There is no data available, however, to show how long one would have to decrease fat intake to reduce cancer risk (Carroll, 1986).

Experimental diets high in fat (>100 g/kg) have been shown to enhance tumorigenesis in spontaneous, carcinogen-induced, X-irradiation-induced and transplantable mammary tumours in both rats and mice (Welsch, 1987). Effects of high fat diet are most marked when fed after initiation and are equally marked in carcinogen-induced and transplantable tumour models. Early experimental feeding studies using the carcinogen-induced model in the rat provided evidence that diets high in polyunsaturated fatty acids were more potent in enhancing tumour growth rates than high saturated fat

diets (Carroll and Khor, 1971). Ip (1987) found that tumour incidence was linearly related to dietary linoleate content up to a maximum level which lay between 4% and 5%, but once this level was reached, the amount of fat fed was the determining factor in tumour incidence. Long-chain polyunsaturated fatty acids seem to be protective even at low dose levels and their protective action remained even when the total fat content of the diet was high (Karmali, 1987). These observations also have implications for human dietary studies and show the need for accurate quantification of dietary fatty acid intakes in different populations.

It has been argued that tumour-promoting effects of high fat diets in both mammary and colon models could be due to the energy content of high fat diets, rather than to an effect of fat *per se* (Kritchevsky *et al.*, 1984). Studies on mammary tumorigenesis using isocaloric diets with varying amounts of fat fed to animals showed that tumour incidence and growth was greater in animals fed high fat diets and lowest in animals fed low fat diets, but equivalent amounts of energy (Hopkins and Carroll, 1979; Cohen *et al.*, 1984). Ip (1987) also showed that animals fed a high fat energy restricted diet, severe enough to impair weight gain, still developed significantly more tumours than animals fed on a standard diet *ad libitum*.

In contrast to the above studies, some others have found that incidence and growth rates of induced tumours are higher in rats fed on low fat, high energy diets than in animals fed high fat, energy restricted diets. Boissonneault *et al.* (1986) fed three groups of rats in whom mammary carcinogenesis was initiated. One group was fed a high fat diet (60% energy from fat) *ad libitum* and another a low fat diet (10% energy from fat), also fed *ad libitum*. The third group was fed a high fat diet under conditions of very modest calorie restriction of about 15%. For the rats fed the high fat diet under modest conditions of restriction the calorie consumption was reduced, but the animals weighed the same as those fed the low fat diet *ad libitum*. Their body composition was more like the rats fed the high fat diet *ad libitum* and the tumour incidence was only 7% compared to 73% (high fat diet *ad libitum*) and 43% (low fat diet *ad libitum*), even though these animals ate three times as much fat as the low fat *ad libitum* fed group and almost as much fat as the high fat *ad libitum* fed group. Hence, the total calorie intake was more important than total fat intake. There is now a notable shift in thinking among scientists

away from concentrating on dietary fat *per se* in favour of more encompassing lifestyle issues like total energy intake *versus* expenditure (Pariza and Simopoulos, 1987).

1:12:1 Breast Cancer

Breast cancer is the most common site of cancer in women in the U.K. and is a major cause of death in women under the age of 65 years (Registrar General, 1987). Although the number of breast cancer deaths is greatest in women over the age of fifty, it accounts for approximately one-fifth of all deaths in women aged 35 to 50 years. Many women over the age of 50 dying from breast cancer developed the disease at an earlier age.

International comparisons show a strong relationship between fat intake and breast cancer rates (Armstrong and Doll, 1975) which is linear with a correlation coefficient of 0.8 to 0.9 (Gray *et al.*, 1979). Prentice *et al.* (1988) showed that the strong international correlation with breast cancer rates holds for total energy from fat but not for non-fat calories. Migration studies indicate that breast cancer rates change toward those of the country to which women migrate. For example, Japanese women in Hawaii have increasing breast cancer rates compared to rates in Japan and the incidence in second generation Japanese women in Hawaii is similar to that for whites in Hawaii (Dunn, 1977). A similar increase in the incidence of breast cancer has been found in Italian women migrating to Australia. Dietary acculturation, including adoption of a diet higher in fat content than that of the country of origin, is a possible explanation for the change in breast cancer rates. Time-trend studies have also revealed a relationship between increase in fat intake and breast cancer rates. In Japan, mean *per capita* daily fat intake rose from 23 g/day in 1957-59 to 52 g/day in 1973 and breast cancer mortality rose approximately by 30% with the sharpest increase (>50%) in women aged 45-54 years (Hirayama, 1978; Kurihara *et al.*, 1984). From the above international studies there appears to be a strong link between fat consumption and incidence of breast cancer.

However, studies of individuals within the same country have yielded conflicting results, perhaps due to the presence of a confounding environmental factor that is associated with dietary fat intake and may be the real causative agent in breast cancer. In a prospective study of 90,000 nurses in the U.S., Willet *et al.* (1984) concluded that there was no relationship between fat intake and breast cancer. However, the lowest quintile of fat intake in this study was 32% of the energy, well above the level of 20-25%

energy seen in low risk countries such as Japan. A recent large-scale case-control study conducted in Italy (Toniolo *et al.*, 1989), where the range of fat intake was wider (lowest quintile 26% energy, highest quintile 46%) showed a significant increase in risk associated with fat consumption of odds ratio 2.1 for total fat and 3.0 for saturated fat.

Dietary fat is strongly associated with breast cancer risk in animal experiments, the effect being promotional leading to increased incidence and multiplicity of tumours. Possible biological roles of fat in breast cancer are listed below in Table 1:6.

TABLE 1:6

Role of dietary fat in breast cancer

1.	Increased production of oestrogens in adipose tissue
2.	Early menarche induced by body fat levels
3.	Late menopause induced by adiposity
4.	Absorbable oestrogen production in bowels affected by intestinal flora
5.	Prostaglandin synthesis
6.	Immunosuppression due to high PUFA intake
7.	Increased epoxide or peroxide levels in breast fluid
8.	Metabolism of chemical carcinogens
9.	Effects of fatty acids on membrane permeability
10.	Interruption of cell-to-cell communication.

Source: Kolmeier *et al.* (1990).

The above table lists various underlying mechanisms through which dietary fat may influence the development of breast cancer, but obesity or adiposity, alterations in hormones and changes in membrane lipids are the main factors.

Body Weight - Obesity

A large-scale study in the U.S. (Lew and Garfinkel, 1979) has shown mortality rates from cancer to be elevated in individuals who were 40% or more above average weight. Colon and rectal cancers were found in excess amongst overweight men, whilst cancers of the reproductive tract, gall bladder and the breast were commonly observed in overweight women. There is a strong body of evidence to support an association between overweight and hormone-dependent cancers in women (La Vecchia *et al.*, 1982). A number of case-control studies have demonstrated a relationship between obesity and risk of breast cancer (Paffenbarger *et al.*, 1980; Helmrich *et al.*, 1983) but some have found only weak association when the analysis was corrected for height (de Waard, 1975; Soini, 1977). The relationship between body weight and breast cancer risk appears to operate only for cancers presenting during post-menopausal years, since an inverse association has been demonstrated for the premenopausal disease. Willet *et al.* (1985), in a study of 120,000 nurses, showed an inverse relationship between BMI and breast cancer risk and this association was strengthened when weight at 18 years of age rather than current weight was considered.

The mechanisms by which overweight may enhance risk of breast cancer in post-menopausal years, but provide protective effects in pre-menopausal years are not known, but it is generally agreed that effects of overweight in post-menopausal women are mediated through increased adiposity and increased capacity for peripheral synthesis of oestrogens in adipose tissue. Excessive weight gain in adult life may be associated with increased risk of post-menopausal breast cancer (Lubin *et al.*, 1985; Ingram *et al.*, 1990). Ingram *et al.* (1990) showed that women who gained more than 10 kg from early womanhood had a two-fold increased risk of developing breast cancer. Obese breast cancer cases have been shown to have more advanced disease on diagnosis and higher rates of recurrence and ^{lower} survival (Rosen *et al.*, 1977; Donegan *et al.*, 1978), perhaps due to the difficulty in detecting tumours in early stages of growth in the obese women. The effect of body weight is more marked in women with oestrogen-receptor-positive than in oestrogen-receptor-negative tumours and this supports the view that hormonal factors underlie effects of bodyweight on breast cancer prognosis (Verreault *et al.*, 1988). Adiposity could lead to alterations in oestrogen metabolism which is believed to be the underlying feature in mammary tumorigenesis (Simopoulos, 1987). The above evidence appears to support the view that the effect of fat is due to increased provision of energy

rather than to a specific effect of dietary fatty acids, and may be consistent with the evidence from animal studies. However, a distinction needs to be drawn between the two, since in experimental animals protective effects of energy restriction appear to operate through growth restriction, whilst in humans harmful effects of high energy diets seem to be related to increased adipose tissue deposition during adult life.

Alterations in Hormones in Response to Dietary Fat Intake

Animal studies provided evidence that high fat diets provoke hypersecretion of prolactin and oestrogens (Chan *et al.*, 1975), but when prolactin secretion was inhibited the differences in tumour incidence in animals fed high and low fat diets disappeared and also ovariectomy has little effect. When high levels of prolactin were induced in animals, the tumour incidence was still high in the high fat group (Ip, 1980), suggesting that the high fat diet was influencing the response to, rather than the secretion of, prolactin. Some studies have failed to observe stimulatory effects of high fat diets on prolactin or oestrogen secretion in rodents (Roger and Westel, 1981; Aylsworth *et al.*, 1984). However, Hill *et al.* (1980) suggested modulatory effects of the level of dietary fat on serum prolactin and oestradiol concentrations. Some studies have failed to demonstrate such effects, but Williams *et al.* (1989) showed a small reduction in luteal phase oestrogens in women transferring from a low to a high fat diet. Conversely, Haggerty *et al.* (1988) failed to show a difference in concentrations of pituitary or ovarian steroids in women following high and low fat diets over a period of one month, although this period may have been too short to detect hormone changes. There was no difference in oestrogen, progesterone or prolactin concentrations in vegetarian and non-vegetarian women despite marked differences in consumption of fat, fibre and energy between the two groups (Schultz *et al.*, 1987). The hypothesis for direct effects of high fat diets on mammogenic hormones such as prolactin and oestrogen is not strongly supported by the current evidence available from animal and human studies (Williams and Dickerson, 1990).

Alterations in Membrane Structure and Function

Dietary fatty acid modification produces profound changes in membrane phospholipid fatty acids in a wide range of tissues in both experimental animals and human subjects. Alteration in mammary membrane fatty acid composition has therefore been proposed as a possible locus for the tumour-enhancing effects of dietary fatty acids

(Williams and Dickerson, 1987). Increased cellular membrane fluidity has been shown to occur in response to increased membrane content of PUFA and has also been associated with increased cell division (Berlin *et al.*, 1980; Lai *et al.*, 1980). Cells derived from proliferating mammary tumours have a higher linoleate content than do normal cells (Kidwell *et al.*, 1982). It has therefore been proposed that the malignant mammary cell may be dependent on the availability of linoleate or EFA, to maintain membrane fluidity and there is no evidence of altered membrane fluidity in tumours of animals fed on modified fat diets. However, this hypothesis may be inconsistent with the inhibitory effects of n-3 fatty acids in tumour growth because, like linoleate n-3 fatty acids are also incorporated into membranes and could enhance tumorigenesis.

Alteration in prostanoid formation, secondary to changes in membrane content of fatty acids, particularly those derived from arachidonate, have been shown to be active in tumorigenesis. It has been reported that indomethacin inhibits tumour formation in rats fed on high linoleate diets, and is also a cyclo-oxygenase inhibitor leading to reduced production of arachidonate from linoleate. Therefore the tumour-enhancing effects of dietary linoleate may be attributed to its role as an essential dietary precursor for the synthesis of membrane arachidonate. This hypothesis is also consistent with reported inhibitory effects of n-3 fatty acids on mammary tumorigenesis since these fatty acids are known to inhibit arachidonate metabolism (Karmali, 1987) and prostanoids derived from n-3 fatty acids are less biologically active than those from arachidonate.

These reports are of interest as epidemiological studies have shown a rising incidence of breast cancer in Greenland, Iceland and Japan during a time when dietary habits have shifted from a high fish, low saturated fat diet to one which closely resembles the Western diet (Nielsen and Hansen, 1980). None of the case-control studies reported so far has measured dietary intakes of n-3 and n-6 fatty acids, but Lubin *et al.* (1981) have reported that a high consumption of fish was associated with reduced risk of breast cancer. Inhibitory effects of high PUFA diets on immune response were associated with higher rates of carcinogen-induced tumour incidence or transplanted tumour growth rates, suggesting that dietary fat may modulate the immune defence response to tumour cells in addition to possible direct stimulative effect of fat on tumour cell proliferation. However, such modulatory effects of both amount and type of fat have been observed in

mammary tumour cells grown in culture as well as in the intact animal (Wicha *et al.*, 1979).

1:12:2 Colon Cancer

Large bowel cancer is one of the 'diseases of Western civilization' being common in NorthWest Europe, North America, Australasia and the River Plate area of South America and less common in Africa, Asia and the Andean countries of South and Central America. Within Europe the incidence of the disease is higher in the North and the West than in the South and East. In the U.K. all age incidence rates for large bowel cancer are second only to lung cancer in men and breast cancer in women, the incidence being the highest in Scotland and Northern Ireland and the lowest in the SouthEast of England. There has been little change in the disease in the past few decades in Western countries, although in the developing countries of Asia and Southern Europe the disease is becoming more common, especially in higher income groups. The risk of disease is correlated with socio-economic status but the differences are small in high incidence countries and more evident in countries with a low incidence (Hill, 1986). In Japan, colon cancer incidence has at least doubled since 1960, suggesting an increase with increasing affluence (Muir *et al.*, 1987). However, the age-standardized rates are considerably lower than in the Western countries, pointing to an international association with 'Western lifestyle' rather than industrialization as such (Jensen, 1986).

Variations in incidence of large bowel cancer within a population are known to exist. In general, within a given country, urban rates tend to be slightly higher than rates for rural areas, particularly in agrarian countries such as Poland or Norway. Differences in incidence have been observed in various ethnic groups living in close proximity. There is a two- to three-fold variation between Chinese, Malays and Indians living in Singapore and a three-fold difference between persons with and without Spanish surnames living in the Southern U.S.A. (Waterhouse *et al.*, 1982). In New Zealand, rates in non-Maoris are three to four times higher than in Maoris. Religious groups such as Seventh-Day Adventists who are mainly lacto-ovo-vegetarians have a markedly lower risk than the general US population (Phillips and Snowden, 1985) and similarly low risk was reported in Seventh-Day Adventists in Denmark (Jensen, 1983). Decreased risk of colon cancer in Seventh-Day Adventists may be attributed to their vegetarian lifestyle but a similar decrease in colon cancer risk was observed in Mormons who are non-vegetarians

(Lyon *et al.*, 1980), suggesting the influence of a healthy lifestyle such as refraining from smoking, higher consumption of wholegrain cereals, and legumes which provide dietary fibre known to lower the risk of colon cancer (Burkitt, 1969).

The study of colon cancer in migrant populations shows that international differences may be ascribed to environmental influences. Migration usually tends to be from low incidence areas to those of high incidence and rarely in the opposite direction. Foreign-born residents in the United States, regardless of the incidence of disease in their (mainly European) country of origin, had similar risk to that of native-born Americans (Haenszel, 1961). There was also a shift in risk of colon cancer in Japanese who move from low risk Japan to high risk U.S.A. Israeli Jews retain the rates of their countries of origin, as Jews born in Europe and the U.S. have higher rates compared to Jews born in Africa and Asia (Waterhouse *et al.*, 1982). Much higher incidence rates are recorded among U.S. blacks than among African blacks. Studies of the cancer risk in various generations of Japanese migrants to the U.S. show that the rates approach the high U.S. incidence for colon cancer within the first generation. A convergence of colon cancer incidence rates to those of Australia has been demonstrated for European migrants to that country, irrespective of whether they arrive from high risk Scotland or from low risk Poland, Yugoslavia, Greece or Italy. The rates approach those of Australia the longer these migrants have lived in the country, probably reflecting the effect of acculturation (McMichael *et al.*, 1980).

Although migrant studies have suggested that differences in the incidence of colon cancer are largely due to environmental factors, within any population genetic factors may affect individual susceptibility to the development of cancer. The presence of adenomatous colon polyps, as in the rare genetically-inherited disease called Familial Adenomatous Polyposis, could be considered as precursor lesions and is associated with elevated risk for colon cancer. A somatic mutation in the 'sporadic' form of colo-rectal cancer is presumed to be subject to environmental influences (Solomon *et al.*, 1987). Other established specific risk factors for colon cancer are inflammatory bowel diseases such as ulcerative colitis and Crohn's disease which greatly increase the risk (Shorter, 1985). Incidence of colon cancer increases with age similarly in both high and low risk populations. However, incidence and sub-site of colon cancer appear to differ with sex. For all sub-sites the risk increases with age, but for the right colon (ascending) the female

rates are higher, and for transverse colon similar to the male rates in older age groups. For the left colon (descending or sigmoid) male incidence in older ages is above that of females. The higher female than male rates at younger ages have been related to the occurrence of menopause, and it has been suggested that female sex hormones may play a role in colon cancer aetiology by influencing bile secretion (McMichael and Potter, 1980).

Nutritional factors are strongly suspected of being important in causing colon cancer. Doll and Peto (1981) have suggested that differences in diet may account for 90% of the variation in colon cancer rates among countries, but specific factors have not been established. Several dietary factors, some protective and some suspected to be causal, have been suggested. Protective factors include dietary fibre, β -carotene, sulphur compound in vegetables such as glucosinolates, vitamin C, selenium, calcium and vitamin E, and those suspected to be implicated in the aetiology of colon cancer include dietary fat, particularly from animal sources, and alcohol. Klasky *et al.* (1988) reported higher rates of colon cancer among regular drinkers, especially women, than among non-drinkers. Another study showed a relationship between beer consumption and colon cancer. Brewery workers have been found to have much higher rates of colon cancer. This relationship is not consistent and it has been suggested that the brewing method may be of importance. Lager beers are not associated with increased risk, but other beers such as Guinness are. Beer may contain nitrosamines, which are potent carcinogens, resulting from the fermentation of nitrates in the beer. Among these dietary factors most attention has focused on dietary fibre and dietary fat.

Dietary Fibre and Colon Cancer

Interest in the relation between the intake of fibre and colon cancer derives primarily from Burkitt's observation of low rates of colon cancer in areas of Africa where the fibre consumption and faecal bulk were high. Correlation studies on mortality from colon cancer in 38 countries show that estimated dietary fibre intakes were higher in countries at low risk for colon cancer, with correlation coefficient relating total dietary fibre and cereal fibre consumption to colon cancer death rates of $r = -0.66$ and $r = -0.72$. However, the strongest association between colo-rectal cancer and large bowel cancer epidemiologically is with meat (Armstrong and Doll, 1975), and animal protein intake, and cereal consumption are inversely related worldwide (Perisse *et al.*, 1969).

Hence these relationships are substantially reduced on partial correlation analysis controlling for meat ($r = -0.36$) and fat ($r = -0.18$) (McKeown-Eyssen and Bright-See, 1985). However, colon cancer is uncommon in Eskimos, suggesting that much of the association is related to the fat content of meat from land animals.

Intra-population studies do not suggest that there is a clear association between fibre intake and colon cancer. Age-standardized colo-rectal cancer rates of the Maoris in New Zealand are approximately half of those of New Zealand whites and yet intakes of dietary fibre are virtually similar (Smith *et al.*, 1985). Dietary fibre intakes are also similar in different racial groups in South Africa who are also at very different risk of bowel cancer (Walker *et al.*, 1986). The marked increase in colon cancer in Japan over the past 30 years has not been associated with changes in intakes of dietary fibre (Kuratsune *et al.*, 1986), but there has been a considerable increase in fat and meat consumption along with a marked decrease in rice consumption.

Results of case-control studies reported so far are difficult to interpret as estimation of fibre intakes was not standardized. The accurate methods of assessment of dietary fibre content of foods as non-starch polysaccharides (NSP) have only recently been available (Englyst *et al.*, 1989). High intake of fruit and vegetables has been consistently related to lower risk of colon cancer, but consumption of cereal products has not. Evidence that grain products are protective is considerably weaker than it is for fruit and vegetables when fibre sources were examined separately (Bingham, 1990). Vegetables also contain numerous flavours and colours such as flavonoids, tannins, isothiocyanates, indoles and phenols which have been found to reduce the incidence of some chemically-induced cancer (Wattenburg, 1990). However, in the largest study of 818 cases in Belgium, starch, fibre and vegetable consumption were all protective factors with relative risks for colon cancer being reduced to 0.71, 0.37, 0.82 and 0.67 respectively in the highest levels of cooked vegetables, raw vegetables, starch and fibre consumption. Peas and beans were associated increased risk (Tuyns *et al.*, 1987, 1988). In a large prospective study in Japan, Hirayama (1981) found low intakes of vegetables and cereals in those individuals who developed cancer later. Stampfer *et al.* (1987) reported lower intakes of crude fibre and some vegetables in 141 Seventh-Day Adventists who developed colon cancer compared to those Adventists who did not. No significant

findings for crude fibre or fibre-related foods have so far been found in a prospective study of 88,751 U.S. nurses (Willet *et al.*, 1990).

NSP intake has been related to colon cancer incidence in Britain and Scandinavia, both of which consume high amounts of meat. In Britain the correlation between NSP intake and age-standardized colon cancer mortality rates was -0.74 ($p < 0.05$), but there was no significant association between NSP intake and colo-rectal cancer mortality and the strongest association was with vegetable consumption ($r = 0.94$) (Bingham *et al.*, 1985); whereas in Scandinavia there was a significant negative association between colo-rectal cancer incidence and NSP intake, which was largely accounted for by cereal consumption rather than vegetables (IARC Large Bowel Cancer Group, 1982). Japanese do not consume more NSP than the European population despite their high cereal consumption because of the lower NSP content of rice. However, they appear to be at lower risk for colon cancer. Therefore the effect of resistant and partially resistant starch reaching the large gut may be important (Bingham, 1990).

Possible Mechanisms

It is suggested that NSP and possibly starch interact with meat and other aspects of diet, particularly meat and fat, in affecting risk from large bowel cancer. The colonic flora and the control of its activity by diet play a key role in cancer aetiology. Anaerobic bacteria in the colon have a potential for a variety of metabolic transformations. Enzymes are readily induced by changes in the metabolic environment and a wide variety of substances can be hydrolysed, reduced, degraded or synthesized, which may be important in the generation of carcinogens or in degrading or re-activating carcinogens secreted in bile. The metabolic environment is determined by dietary intakes, particularly protein, fat and carbohydrate. Carbohydrate stimulates anaerobic fermentation in the large bowel leading to an increase in microbial cell mass and production of short-chain fatty acids (SCFA): acetate, propionate and butyrate. Fermentation has a number of consequences in large bowel physiology and possible implications for large bowel cancer. First, the increase in bacterial cell mass results in an increase in faecal weight (Stephen and Cummings, 1980). Cereal fibre from bran and some resistant starch has an additional effect because it is only partly fermented and the residual polysaccharides absorb water, also contributing to faecal bulking (McBurney *et al.*, 1985). With this increase in faecal bulk, transit time is reduced and the contents of the large bowel are

diluted, thus reducing the time putative carcinogens are in contact with large bowel mucosa.

Fermentation may also affect risk of colon cancer via its effect on pH, bile acids and SCFA production. Bile acids are thought to promote carcinogenesis and it is proposed that the conversion of primary to secondary bile acids is a key step in the development of bowel cancer (Thornton, 1981). The SCFA produced during fermentation are thought to lower pH and so inhibit this conversion. Cereal fibre dilutes faecal bile acid content, although the total output may be increased (Cummings *et al.*, 1976). Starch appears to be beneficial as a substrate for fermentation because the yields of the SCFA-butyrate are increased. Butyrate has been postulated to be an energy substrate for the colonic mucosa and an anti-proliferative and differentiating agent in cell culture lines (Bingham, 1990), but this remains a postulate.

The main product of bacterial metabolism of nitrogenous residues is ammonia, which enhances cell proliferation, alters DNA synthesis and is thus implicated in large bowel carcinogenesis (Visek, 1978). When carbohydrate availability is limited, ammonia concentrations increase *in vitro* (Cummings *et al.*, 1979), but in the presence of active fermentation ammonia is used for bacterial protein synthesis and faecal ammonia concentration falls (Macfarlane *et al.*, 1986). Diets high in meat generally also contain little starch and NSP and can therefore be expected to increase ammonia concentration partly by an increase in available nitrogen and because of limited fermentation. Levels of faecal ammonia have not yet been measured. The low incidence of colon cancer among Eskimos and American Indians, who have high intakes of animal protein, indicates that animal protein alone may not be a causative factor. There is clearly an interaction between several dietary factors. The study by Hirayama *et al.* (1985) illustrates this concept. Table 1:7 shows results of the Hirayama study.

TABLE 1:7**Relationship between daily meat or vegetable consumption and colon cancer risk**

Dietary pattern	Colon Cancer Risk (rate/100,000)
<i>Neither meat nor vegetables on a daily basis</i>	<i>14.9</i>
<i>Meat but not vegetables on a daily basis</i>	<i>18.43</i>
<i>Vegetables but not meat on a daily basis</i>	<i>13.67</i>
<i>Meat and vegetables on a daily basis</i>	<i>3.87</i>

*Source: Hirayama (1985).****Dietary Fat and Colon Cancer***

A substantial number of studies have examined the relationship between dietary fat and the risk of colon cancer. Tables 1:8, 1:9 and 1:10 summarise correlation, case-control and prospective studies on colon cancer. A majority of international correlation studies showed a strong positive relationship between dietary fat and colon cancer mortality or incidence in different countries, but these were based on food balance sheets of the country rather than on individual intakes. However, individual intakes do correlate strongly with *per capita* availability. Studies on populations within countries are not consistent with international correlations. Most case-control studies have found a stronger positive association of meat intake and colon cancer risk than of dietary fat. Prospective studies have not shown any clear relationship between dietary fat and colon cancer. The same criticisms have been made of the relationship between dietary fat and CHD. The dietary methodology in most of these studies was inadequate, usually based on food frequency questionnaires or 24-hour recall. Only in one study by Jain *et al.* (1980), actual fat intakes were assessed and an association with saturated fat was found. One problem may be that susceptibility may differ inside exposed populations. Both CHD and colon cancer are associated with high intakes of saturated fat between populations, yet not inside populations. Individuals at risk of CHD tend to have higher levels of plasma cholesterol, whereas low plasma cholesterol concentrations are associated with increased risk of colon cancer (Rose *et al.*, 1974). It can be hypothesised that those individuals who are able to maintain low plasma cholesterol concentrations do so by increasing bile acid and sterol excretion which in turn makes them more susceptible to bowel cancer (Lowenfels, 1983).

TABLE 1:8 Summary of correlation studies of fat and colon cancer

Source	Populations Studied	Association with Dietary Fat	Comments
<i>Draser and Irving (1973)</i>	37 countries	Positive	Also positive for animal fat
<i>Howell (1975)</i>	37 countries	Positive	Positive for meat, especially beef, but not chicken and pork
<i>Armstrong and Doll (1975)</i>	32 countries (mortality) 23 countries (incidence)	Positive	Also positive for meat, animal protein and total energy
<i>Knox (1977)</i>	20 countries	Positive	Also positive for meat, animal protein and total energy
<i>Hirayama (1979)</i>	29 districts in Japan	Positive	Based on meat consumption
<i>McKeown-Eyssen and Bright-See (1984)</i>	38 countries	Positive	Especially animal fat; also positive for red meats
<i>Enstrom (1975)</i>	48 US states	None	Also no association for meat
<i>Bingham et al. (1979)</i>	9 regions in the UK	None	Also no association for meat
<i>McMichael et al. (1979)</i>	4 countries	None	Based on time-trends; also no association with meat
<i>Lyon and Sorensen (1978)</i>	Mormons/non-Mormons in Utah	None	No association for meat
<i>Kinlen (1982)</i>	Catholic nuns in the UK	None	No association for meat
<i>Kolonel et al. (1981)</i>	Polynesians/Caucasians in Hawaii	None	
<i>Smith (1985)</i>	Maoris/non-Maories in New Zealand	None	
<i>Kolonel et al. (1986)</i>	Japanese migrants in Hawaii	None	Positive association for protein

TABLE 1:9 Summary of case-control studies of dietary fat and colon cancer

Source	Sample Size and Country	Association with Dietary Fat	Comments
<i>Jain et al. (1980)</i>	348 cases & 542 matched controls (Canada)	Positive	Saturated fat in particular; also positive for total energy and protein
<i>Miller et al. (1983)</i>	Same as above	Positive	No association with individual foods
<i>Wynder et al. (1969)</i>	69 cases & 307 hospital controls	-	Positive for total energy
<i>Haenszel et al. (1973)</i>	179 cases & 357 hospital controls - Japanese migrants in Hawaii	-	But positive for beef
<i>Dales et al. (1979)</i>	99 cases & 280 hospital controls - American blacks	dose response gradient of increased risk	Weak association in males only with high fat foods
<i>Pickle et al. (1984)</i>	58 cases & 116 matched controls - Rural Nebraska	Positive, but not significant	Also positive for meat and dairy products
<i>Modan et al. (1975)</i>	198 cases & 510 controls - Israel	-	No association for meat
<i>Manousos et al. (1983)</i>	100 cases and 100 controls - Greece	-	Association with meat
<i>Potter and McMichael (1986)</i>	220 cases & 732 controls - Australia	-	Increased risk with energy intake and protein
<i>Macquart-Moulin et al. (1986)</i>	196 cases and 399 controls - France	-	
<i>Lyon et al. (1987)</i>	246 cases & 484 controls - USA	None	Increased risk with increasing total energy consumption
<i>Thyris et al. (1987)</i>	453 cases & 2851 controls - Belgium	None	
<i>Kune et al. (1987)</i>	392 cases & 727 controls - Australia	Positive	

TABLE 1:10 Summary of prospective studies on colon cancer

Source	Sample Size and Country	Association with Dietary Fat	Comments
<i>Stemmerman et al. (1984)</i>	106 cases/7074 men of Japanese origin in Hawaii	<i>Inverse</i>	<i>Especially with saturated fat</i>
<i>Hirayama (1981)</i>	265,118 adults in Japan	<i>Inverse</i>	<i>Protective against colo-rectal cancer mortality independent of green and yellow vegetable intake</i>
<i>Garland et al. (1985)</i>	29 cases in Chicago	<i>None</i>	<i>No association with percentage energy from fat or protein</i>
<i>Phillips and Snowden (1985)</i>	25,493 Seventh Day Adventists	<i>None</i>	<i>Positive association with egg consumption - likely effect of cholesterol</i>
<i>Jenson et al. (1980)</i>	Spouses of 1716 cases	<i>None</i>	<i>No effect of dietary fat on colon cancer risk in spouses</i>
<i>Willet et al. (1990)</i>	88,751 nurses in the USA	<i>None</i>	<i>Increased risk with daily consumption of beef, pork or lamb and decreased risk with consumption of chicken and fish</i>

Both the quantity and quality of fat in the diet appears to affect colon carcinogenesis. Increasing fat intakes promotes growth. However, much of this effect can be abrogated by energy restriction. Several animal model studies have demonstrated that high fat diets containing corn oil sunflower oil, lard and beef tallow enhanced the appearance of chemically-induced colonic tumours in rats, whereas diets containing high levels of coconut oil, olive oil or *trans* fatty acids had no colon tumour-promoting effects (Bull *et al.*, 1981; Reddy and Maeura, 1984; Reddy *et al.*, 1985). Increasing intakes of linoleic acid promote tumour growth independently of total fat intake (Reddy and Maeura, 1984) and this effect can also be abrogated by indomethacin, implying that promotion is mediated via prostaglandins (Balch *et al.*, 1982). Fish oils rich in n-3 polyunsaturated fatty acids have been shown not to enhance azoxymethane-induced carcinogenesis in the rat (Reddy and Maruyama, 1986). The excretory pattern of faecal secondary bile acids which have been shown to act as large bowel tumour promoters, are positively correlated with large bowel tumour incidence in animal models fed various types and amounts of dietary fat (Reddy, 1983). It is likely that fish oils may have an inhibitory effect on the colonic concentration of secondary bile acids. This suggests that it is the type of polyunsaturated fatty acids that is of importance, rather than the general level of fat in the diet.

Mechanisms

There is clear evidence from experimental studies that dietary fat can promote carcinogenesis. However, it needs to be considered that many carcinogens are fat-soluble and so fat could act as a vehicle for dietary carcinogens such as nitrosamines. Increasing the intake of dietary fat increases the secretion of bile acids. Gut microflora metabolize these and neutral sterols into co-carcinogens in the colon (Hill *et al.*, 1971). Studies of faecal steroid excretion have found lower concentrations of total and secondary bile acids in populations at low risk of colon cancer compared with those at high risk. Populations consuming a high fat diet appear to have higher levels of faecal bile acids (Table 1:11).

TABLE 1:11

Bile acid concentrations in faecal samples from populations at different risk of developing colon cancer

Population	Total Faecal Bile Acids*	Deoxycholic Acid*	Incidence of Colon Cancer**
Uganda	0.5	0.1	0.6
Japan	1.4	0.5	4.7
India	0.5	0.3	5.7
South Africa (black)	2.6	1.0	8.6
Hong Kong (Group C)***	2.2	0.9	7.2
Hong Kong (Group B)	3.1	1.2	11.2
Hong Kong (Group A)	4.7	1.7	13.3
England	6.2	1.5	18.2
South Africa (white)	5.9	1.6	19.4
United States (black)	6.3	-	28.0
United States (white)	6.0	2.2	28.1
Scotland	6.2	2.5	31.2
Kuopio (rural Finland)	7.8	-	5.5
Parrikala (rural Finland)	6.3	-	6.7
Them (rural Denmark)	8.0	-	12.9
Helsinki (urban Finland)	7.8	-	17.0
Copenhagen (urban Denmark)	8.4	6.0	20.7
Copenhagen (urban Denmark)	9.5	-	22.8
Greenland (Eskimos)	7.7	5.5	19.0

* mg/g of dry faeces

** cases per 100,000 population

*** Hong Kong population sub-groups divided by income

Source: Thompson (1985).

Bile acid degradation to secondary substrates is also apparently reduced in populations consuming a low animal fat and protein diet which is also high in fibre. Vegetarian Seventh Day Adventists in California had lower faecal concentrations of cholesterol metabolites (coprostanol and coprostanone) and secondary bile acids formed in the colon by bacterial action compared with a matched group of non-vegetarians in the general population (Turjman *et al.*, 1984). The concentrations of both total faecal bile acids and neutral sterols were significantly higher in the non-vegetarians (Nair *et al.*, 1984). Asians (vegetarian and non-vegetarian) were reported to excrete significantly lower concentrations of secondary bile acids and neutral sterols (McKeigue *et al.*, 1989). Both international and intra-population studies show that bile acid excretion in faeces differs with the nature of the diet and colon cancer risk.

1:13 Conclusion

Vegetarianism is generally believed to afford some protection against 'Western Diseases' such as CHD and cancers, particularly due to the modified fat and fibre content of the diet. Indian migrants living in host countries (East or West), despite their predominant vegetarian dietary habits, suffer from higher incidence of, and mortality from, CHD while having low mortality from cancers. In international comparisons, national mortality rates from CHD and cancer, especially of breast and colon, are correlated with each other and with the intake of dietary fat. However, a dissociation of these two diseases is seen in the Indians and they as a group could provide a unique opportunity to study the aetiology of these diseases. On the other hand, a vegetarian diet, while providing some protection against CHD in the Caucasian population, does not appear to offer similar benefits to the Indians and most of these assumptions were based on studies conducted in males. It has also been observed that women tend to adhere to the vegetarian diet far more strictly compared to the male members of the family. Therefore, the present study was undertaken firstly to characterize the diets of Indian (IV) and Caucasian vegetarian (CV) women and to compare them with the Caucasian omnivorous (CO) diet. The second objective is to study the influence of their respective diets on markers of disease:

- Nutritional anaemias through haematological studies
- Coronary heart disease risk through blood lipoprotein profiles
- Breast cancer risk through sex hormone levels in plasma
- Colon cancer risk through faecal profile of bile acids and neutral sterols
- Birth weights of Hindu vegetarians are compared with Caucasian controls.

CHAPTER 2

METHODS

2:1 Selection of Subjects

2:1:1 Indian Vegetarians and Caucasian Omnivores

The London Borough of Brent was chosen for the study, as the ethnic minority population in the borough predominantly comprises of vegetarian Gujaratis of Indian origin. Approval for the study was obtained from the Ethical Committee of the Brent Area Health Authority. Indians and Caucasians were drawn from general practice lists at Craven Park Health Centre in Harlesden, North-West London and a group practice in Wembley, North London. Both the selected practices were within a mile of each other thereby ensuring that the subjects were drawn from similar socio-economic background. Lists of all women patients aged 25-40 years were obtained from the two general practice lists. The Indian women were selected based on their Gujarati surnames. Introductory letters, as well as a brief description of the study, were sent to all the Gujarati women and a random sample of Caucasian women. An initial questionnaire (Appendix 1) was enclosed to ascertain: ethnic origin, age, sex and dietary practice. The subjects were also asked to indicate their willingness to participate in the study and return the questionnaire using the pre-paid envelope enclosed.

Craven Park Health Centre was a large group practice and lacked regular updating of patient addresses. This is reflected in the high number of letters (19) returned due to incorrect address (Table 2:1). In contrast, none of the letters sent to patients from the Wembley surgery were returned. However, the 'final' response rate in the Indians was similar (41.1% and 41.4%) in both practices but response from Caucasians drawn from the Wembley practice was better when compared to Craven Park.

2:1:2 Caucasian Vegetarians

Ideally this group should have been drawn from the same area as the other groups, but this was not feasible. Although vegetarianism has been gaining in popularity among Caucasians, the proportion of vegetarians was very low in the patients from both general practice lists. Therefore, Caucasian vegetarians were recruited with the aid of the London branch of the Vegetarian Society, Barley Mow Passage, Chiswick. An advertisement for female volunteers aged 25-40 years living in the inner London area was placed in "The Vegetarian", a magazine published by the Vegetarian Society. Respondents to the advertisement were sent details of the study and questionnaire (Appendix 1). All the respondents except one (due to pregnancy) were accepted into the study.

TABLE 2:1 Responses to questionnaire

	Total No. Sent	Total No. Returned	No. Returned Incorrect Address	No. of Respondents Unsuitable*	No. Accepted	No. of Refusals**	Total Response (%)
<u>Indians</u>							
Craven Park	73	36	6	4	15	11	41.1
Harrow Road, Wembley	56	32	none	2	19	11	57.1
<u>Caucasians</u>							
Craven Park	58	37	13	3	16	5	41.4
Harrow Road, Wembley	45	27	none	none	8	19	60.0

* Includes pregnant women, Indian non-vegetarians and West Indians, and two male subjects.

** Includes those subjects who withdrew from the study after a full description of the protocol.

2:1:3 Criteria for exclusion from the study

Any volunteers who were taking steroids (other than oral contraceptives), antibiotics or receiving treatment for diabetes and hypertension were excluded. Pregnant and lactating women and those on any special diets were also excluded.

2:2 Protocol of the Study

All the subjects who agreed to participate in the study were visited at home. Detailed general, health and dietary information was obtained by interview and completion of questionnaires (Appendices 2 and 3). This helped in building a rapport with the subjects and ensured their compliance through the various stages of the study. The date of last onset of menstrual period was ascertained. Appointments were made for the subjects to attend their doctor's surgery for phlebotomy and anthropometry and Caucasian vegetarians were asked to report to the Department of Nutrition, King's College, London. It was feasible to have three subjects per week participating simultaneously in the study. In order to reduce seasonal effects on the results, it was ensured that subjects from three groups were represented each of the weeks, during which the study was conducted.

2:2:1 Dietary Information

Nutrient intakes of the subjects were assessed by conducting a Seven Day weighed dietary intake survey. Each subject was provided with a food recording kit consisting of standardised dietary scales (Soehnle Digita, West Germany), food diary and detailed instructions on how to record their food intake. They were also asked whether they took any dietary supplements. An effort was made to obtain dietary intake to include days of both follicular and luteal phases of the menstrual cycle. The body weights of the subjects were recorded before and after the period of dietary record. Caucasian subjects were visited twice during the week but as the Indians required more supervision they were visited about four times. Additionally, the subjects were asked to keep a weighed record of individual recipes of foods cooked at home. Samples of cooking fats and oils used in the individual households were also collected and frozen at -20°C until analyzed for their fatty acid content.

2:2:2 Phlebotomy

The subjects attended their doctor's surgery after an overnight fast of at least ten hours. Forty millilitres of blood were collected by venepuncture into evacuated tubes with and without anti-coagulant (EDTA). Plasma and serum were separated by centrifugation and frozen at -20°C until analyzed. Plasma (2.5 ml) was kept refrigerated for the separation of total HDL and HDL₃ cholesterol fractions. Five millilitres of whole blood were despatched on the same day to the Haematology department of the Central Middlesex Hospital, Acton, London NW10.

2:2:3 Anthropometry

The height of the subjects in centimetres was measured by a fixed Harpenden Stadiometer (British Indicators Ltd). Their weight in kilograms was recorded using beam balance (CMS Weighing Equipment Ltd). Harpenden Callipers (British Indicators Ltd) were used to measure skinfold thickness at four sites: biceps, triceps, sub-scapular and supra-iliac as described by Durnin and Rahaman (1967). Waist and hip circumferences in centimetres were recorded according to Ashwell *et al.* (1982). Percentage body fat was estimated according to the data of Durnin and Womersley (1974). In order to avoid variation all the measurements were made by the same technician throughout the study.

2:2:4 Blood Pressure

Systolic and diastolic pressures were measured with Random Zero Sphygmomanometer (Hawksley and Sons Ltd).

2:2:5 Collection of 24 hour Urine and Faecal Samples

Subjects were required to collect all the urine and faeces excreted in 24 hours on any one day of the week in which they were recording their food intake. Urine was collected in a plastic container of 3 litre capacity containing 10 ml of acetic acid and toluene (1:1). A faecal collection kit consisting of a metal frame fitted with a collection box, which could be placed on the toilet was provided. The contents were kept chilled in a insulated box with cool packs until collected and transported to the laboratory. Detailed instructions on use of the kit and 24 hour collection were also included. Faeces was stored in the collection boxes at -20°C until analyzed for bile acids and neutral sterols.

2:3 Assessment of Nutrient Intakes

Seven day food intake records obtained from the subjects were analyzed by 'Foodtabs' a computer software programme (T.A.B. Sanders, unpublished), based on the McCance and Widdowson's Food tables of Paul and Southgate (1978) and supplements (Tan *et al.*, 1985; Holland *et al.*, 1988). Other sources of food composition data were used for foods not included in that database, such as information provided by the manufacturers and U.S. Food Tables (United States Department of Agriculture, 1979). Some of the Indian foods, especially snack foods, were analyzed for their nutrient composition and data included in the database. In order to estimate fatty acid intakes as accurately as possible, fatty acid composition of individual household fats were incorporated into their respective food records.

2:4 Analytical Methods

2:4:1 Analysis of Household Fats and Oils

Methyl esters of fatty acids were prepared by the method of Christie (1973). Fats solid at room temperature such as butter and margarine were prepared for methylation by dissolving 100 mg of fat in 2 ml of hexane puriss. Anhydrous Sodium sulphate (0.5 g) was added and shaken and 0.3 ml aliquot of the supernatant was taken for methylation. In the case of an oil, 20 μ l was dissolved in 0.3 ml hexane prior to methylation.

Methylation: 0.1 ml 2N sodium methoxide, prepared by dissolving sodium metal in dry methanol was added to 0.3 ml supernatant or oil dissolved in hexane. The mixture was shaken gently for five minutes and 2.5 ml hexane was added, followed by a small amount of anhydrous calcium chloride. The sample was allowed to methylate for at least one hour at room temperature or overnight at 4°C, then centrifuged and an aliquot of the supernatant could then be taken for analysis by capillary Gas Liquid Chromatography.

2:4:2 Analysis of Fatty Acids from Foods

Foods Analyzed

Aundhavo	Kachori
Bhajia	Puri
Cassava chips	Thepla
Farsi puri	

Samples of the above foods were obtained from take-aways and cafés in the Wembley area. Each food was collected from six different sources and homogenised. The homogenate was used for fatty acid analysis by the method of Roshanai and Sanders (1984). Ten grams of homogenate were extracted with a mixture of chloroform : methanol (1:1) by volume with vigorous agitation. To 10 ml of extract 2 mg penta-decanoic acid (15:0) in 1 ml methanol was added as an internal standard. The lipid extract containing the standard was dried under a jet of nitrogen and dry lipid methylated.

Methylation by boron trifluoride (Morrison and Smith 1964): Dry lipid was methylated by a mixture of 14% BF₃ : methanol : benzene (35:30:35 by volume). Two millilitres of the mixture were added to dry lipid, the tubes flushed with nitrogen, capped and heated in a boiling water bath for 45 minutes. The tubes were cooled and 4 ml hexane and 2 ml water were added. The upper phase of hexane containing methyl esters was collected and dried under a jet of nitrogen at 50°C. Dry methyl esters were redissolved in 100 µl hexane and placed on Sep-Pak silica cartridges (Millipore: Waters Associates, Milford, U.S.A.) for purification. The esters were eluted with 5 ml mixture of hexane and diethyl ether (9:1). The eluate was evaporated under nitrogen and dry lipid was redissolved in 0.8 ml hexane. An aliquot was used for analysis by capillary Gas Liquid Chromatography.

2:4:3 Haematology

Full blood counts were made on a Coulter counter and examined by the Haematology department of the Central Middlesex Hospital.

Serum folate and Vitamin B₁₂ were determined in frozen serum simultaneously by Simultrac Radio-Assay kit supplied by Becton Dickinson and Co, New York, U.S.A.

Erythrocyte folate was determined by incubating fresh whole blood with ascorbic acid which lyses erythrocytes and liberates folic acid which is also reduced by ascorbic acid. Folic acid in haemosylate was determined by radio-assay as for serum folate.

Serum Ferritin was determined by (¹²⁵I) Radio-Immunoassay Kit (Becton Dickinson Immunodiagnosics, New York) in frozen serum samples.

2:4:4 Plasma Lipids

Total Triglycerides: 20 µl of fresh plasma was taken and analyzed by Peridochrom Triglycerides GPO-PAP; Boehringer Mannheim kit. The method involved complete hydrolysis of triglycerides and subsequent determination of the liberated glycerol by colorimetry by the use of Adenosine tri-phosphate (ATP).

Total Cholesterol: Plasma cholesterol was determined by the CHO/PAP method (Boehringer Mannheim, Lewis, Sussex). This is an enzymatic colorimetric method involving the hydrolysis of cholesterol esters to produce free cholesterol which reacts with cholesterol oxidase to produce hydrogen peroxide, which in turn reacts with 4-amino phenazine and phenol. The colour thus developed was measured colorimetrically.

Total HDL-Cholesterol and Fractions: Cholesterol fractions were separated by sequential precipitation (Gidez *et al.*, 1982). Total HDL was separated by precipitating other plasma lipids with manganese chloride. An aliquot of the supernatant was further treated with dextran sulphate to precipitate HDL₂-Cholesterol. Both supernatants containing total HDL and HDL₃ fractions were treated with HCO₃⁻ (Bachorik *et al.*, 1984) and cholesterol was determined by the method described above. The HDL₂ fraction was estimated by the difference in total HDL-Cholesterol and HDL₃-Cholesterol.

LDL-Cholesterol: The formula of Friedwald (1972) was used to estimate LDL-cholesterol in plasma:

$$\text{LDL-Cholesterol (mg/dl)} = (\text{Total cholesterol}) - (\text{Triglycerides}/5) - (\text{HDL-Cholesterol}).$$

Apoprotein A1 and Apoprotein B: Frozen plasma was analyzed for Apo A1 and Apo B quantitatively by Apolipoprotein A1 and B kits supplied by Orion Diagnostica, Espoo, Finland. Both methods were based on immuno-precipitation by the specific antiserum and the turbidity of the solution measured at 340 nm in a Pye Unicam Spectrophotometer.

Apolipoprotein(a): Apo(a) was determined in frozen serum samples by Radio-Immunoassay (Pharmacia Diagnostics AB, Uppsala, Sweden). This method is a two site immunoradiometric assay (two site IRMA) using two different monoclonal antibodies in

excess. During incubation Apo(a) in the sample reacts with anti-Apo(a)-¹²⁵I antibodies and anti-Apo(a) antibodies attached to solid phase, micro-sepharose. The formed antibody-antigen complex is separated from excess tracer by addition of decanting solution followed by centrifugation and decanting. The radio-activity of the pellet is measured and is directly proportional to the concentration of Apo(a) in the sample.

Plasma Free Fatty Acids and Phospholipid Fatty Acids: Free fatty acids and phospholipids in plasma were simultaneously separated from plasma lipid by the method described by Kaluzny *et al.* (1985). To 1 ml of plasma 2.5 ml internal standard containing 3 mg pentadecanoic acid and 10 mg di-heptadecanoyl phosphatidyl choline in 100 ml of methanol was added. Lipid was extracted from the mixture with methanol initially and later with chloroform. The chloroform fraction containing lipid was dried under a jet of nitrogen. The dry lipid was redissolved in 250 µl chloroform. Lipid fractions were separated by using Bond Elut Aminopropyl disposable columns (Analytichem International, Harbor City, U.S.A.). Aminopropyl columns were placed in the vac-elut apparatus and pre-washed with hexane. Plasma lipid in chloroform was applied to the column and chloroform eluted under vacuum while the entire lipid mixture was left on the column. Different classes of lipids were separated by sequential elution by various solvents as follows:

A	Chloroform-2-propanol (2:1)	-----	all neutral lipids
B	2% Acetic acid in diethyl ether	-----	free fatty acids
C	Methanol	-----	all phospholipids

Eluates B and C were collected in separate tubes and solvents evaporated under a jet of nitrogen and the dry lipid was redissolved in hexane. Free fatty acids and phospholipid fatty acids were methylated by the boron trifluoride method described earlier and methyl esters were analyzed by capillary Gas Liquid Chromatography.

Total Esterified Fatty Acids in Plasma: Fatty acids in all classes of lipids were directly esterified by the method described by Lepage and Roy (1986). Pentadecanoic acid (15:0, 50 µg/ml of a mixture of methanol and toluene [3:1] to which 10 ml of acetyl chloride were added) was used as an internal standard. The internal standard mixture (2.1 ml) was added to 100 µl of plasma. The test tube was flushed with nitrogen, capped and

heated in a boiling water bath at 100°C for one hour. Five millilitres of 6% potassium carbonate were added to the tubes after cooling and vortex mixed. The tubes were centrifuged to separate the upper layer of the toluene containing fatty acid esters which were analyzed by Gas Liquid Chromatography.

2:4:5 Retinol and α -Tocopherol in Plasma

Retinol and α -tocopherol status of the subjects was assessed by simultaneous measurement of their concentrations in plasma (Bieri *et al.*, 1979; Driskell *et al.*, 1982). The vitamins were extracted from 500 μ l of plasma with a mixture of ethanol and hexane (1 ml) in the presence of an internal standard (500 μ l) of retinyl acetate (100 μ g/dl). The contents of the tube were vortex mixed and centrifuged at 1000 rpm for five minutes. The upper phase was collected and solvents removed under a jet of nitrogen. The dry lipid was redissolved in 250 μ l ethanol. A set of external standards containing all *trans* retinol ranging from 10-100 μ g/dl and α -tocopherol ranging from 0.4-2.4 mg/dl were prepared similarly to the samples. The samples and standards were analyzed using a 25 cm reverse phase C₁₈ HPLC column attached to a ultra violet detector (290 nm, 0.01 FSD) and an electronic integrator (PW 5). Aliquots (50 μ l) were injected onto the column using a microsyringe and UV absorption was measured relative to the internal standard. A standard curve of the ratio of peak areas of internal standard and retinol or α -tocopherol were plotted and used to determine the concentration of retinol and α -tocopherol in plasma samples.

2:4:6 Sex Hormones in Plasma

Frozen plasma samples were analyzed for sex hormones and sex hormone binding globulin at the Imperial Cancer Research Fund Laboratories (ICRF), Lincoln's Inn Fields, London.

Sex Hormone Binding Globulin (SHBG) was measured with an immuno-radiometric assay kit (Farnos Diagnostica, Oulunsalo, Finland).

Total Testosterone (T) concentration in plasma was determined with a non-extraction double antibody radio-immunoassay kit (Sorin Biochemia U.K. Ltd, Ely, U.K.).

Free Testosterone (Free T) was estimated by the formula of Nanjee and Wheeler (1985).

$$\% \text{ Free T} = 6.11 - 2.38 * \log_{10} \text{SHBG}$$

Total Oestradiol (E₂) was measured with a charcoal separation radio-immunoassay kit (ER-150, Steranti Research Ltd, St. Albans, U.K). The method involved sample extraction into ether prior to assay.

Free Oestradiol (Free E₂): Non-protein bound oestradiol was estimated from the equation of Moore *et al.* (1982).

$$\text{Log free E}_2 = -0.003 \text{ SHBG} + 0.389$$

2:4:7 Urine Analysis

Creatinine in urine was determined by the method of Oser (1965) modified for automated analysis using Newton Digital sampler (The Newton Instrument Company Ltd., Merseyside) and a Technicon AA1 proportioning pump (Technicon Instruments Ltd., Surrey). The yellow red colour produced after reaction with alkaline picrate was measured spectrophotometrically at 525 nm and recorded on a chart recorder. Creatinine standards in the range of 0.25-2 mg/ml were used for calibration.

Sodium and Potassium: An aliquot of 24 hour urine collected by the subjects was analyzed by flame photometry (Dean, 1960). Urine samples were diluted 1:500 in preparation for analysis. Two sets of standards containing 0-10 µg/dl of sodium and potassium and diluted urine samples were read in Corning 410 Flame Photometer using the appropriate filters.

2:4:8 Faecal Analysis

Faeces were analyzed for faecal steroids at Public Health Laboratory Service, Porton Down, Salisbury, Wiltshire. Frozen faecal samples were homogenized and freeze dried. A 0.5 g aliquot of freeze dried sample was sequentially extracted and fractionated by lipophilic anion-exchange chromatography into neutral sterol, free bile acid and

conjugated or sulphated bile acid fractions. Steroid concentrations were determined by Gas Chromatography (Owen *et al.*, 1984).

2:5 Statistical Analysis

Statistical analysis was carried out using SPSS and Minitabs PC statistical packages. Data were analyzed by parametric and non-parametric methods according to the validity of the assumption of normal distribution. One-way and two-way analysis of variance and Duncan's Multiple Range Test were applied for comparison between groups. Pearson's Product-Moment Correlation and Multiple Regression Analysis were used to assess relationships between variables. Qualitative data were assessed using Fischer's Exact Test. The probability value of less than 5% was taken to be significant throughout. Results are expressed as the mean value with standard error of the mean (SEM).

CHAPTER 3

CHARACTERISTICS OF THE SUBJECTS

3:1 Background Information

3:1:1 Age of the Subjects

The age of the subjects on their last birthday prior to commencement of the study was recorded. Omnivores and Indian vegetarians were well matched for age, but the Caucasian vegetarians were younger (Table 3:1).

TABLE 3:1

Age of the subjects in years

Subjects	Mean	SEM	Range
<i>Caucasian Omnivores (n=24)</i>	<i>33.8</i>	<i>0.78</i>	<i>28 - 39</i>
<i>Indian Vegetarians (n=24)</i>	<i>34.5</i>	<i>0.86</i>	<i>26 - 40</i>
<i>Caucasian Vegetarians (n=18)</i>	<i>29.6*</i>	<i>0.91</i>	<i>25 - 37</i>

* $p < 0.05$ significantly different from other groups.

3:1:2 Country of Birth

North and NorthWest London have been traditionally popular residential areas for new immigrants to the UK. All the Caucasian subjects were born in the UK or the Republic of Ireland, with the exception of one Caucasian vegetarian who was born in British territories in the Pacific (Table 3:2). None of the Indian subjects was born in the UK. They had emigrated either directly from India or via Africa (Kenya, Uganda and Tanzania) and one Indian subject was from Yemen. All the Indian women who were born in India had emigrated on marriage to residents of Africa or the UK. The length of residence in the UK of the Indians ranged from 7-21 years.

TABLE 3:2

Country of birth of the subjects (numbers and percentages in parentheses)

Country	CO	IV	CV
<i>UK & Republic of Ireland</i>	<i>24 (100)</i>	<i>0</i>	<i>17 (94.4)</i>
<i>Africa</i>	<i>0</i>	<i>10 (41.7)</i>	<i>0</i>
<i>India</i>	<i>0</i>	<i>13 (54.2)</i>	<i>0</i>
<i>Other</i>	<i>0</i>	<i>1 (4.2)</i>	<i>1 (5.6)</i>

3:1:3 Marital Status

A majority of the omnivores and Indians were married and were well matched for marital status (Table 3:3). Approximately 71% of Caucasian omnivores and 83.3% of Indians were married which was higher than the national averages of 64% and 52% respectively (Central Statistical Office, 1990). Only 33% of Caucasian vegetarians were married, possibly because they were younger than the others. Cohabitation was more popular with both groups of Caucasians and was higher than the national average of 6.3% in 1988 for women aged 25-49 years, and this may be due to the higher cost of living and housing in London. However, it must be noted that small numbers are involved in the present study. Single Indian women prefer to live in parental homes until they are married, as cohabitation may not be socially acceptable. The number of divorced subjects was similar in omnivores and Indians. One Caucasian vegetarian was separated.

TABLE 3:3

Marital status of the subjects (number and percentage in parentheses)

Status	CO (n=24)	IV (n=24)	CV (n=18)
<i>Single</i>	1 (4.2)	2 (8.3)	7 (38.9)
<i>Cohabiting</i>	4 (16.7)	0	4 (22.2)
<i>Married</i>	17 (70.8)	20 (83.3)	6 (33.3)
<i>Separated</i>	0	0	1 (5.6)
<i>Divorced</i>	2 (8.3)	2 (8.3)	0

3:1:4 Number of Children and Household Size

A majority of omnivores and Indians were multiparous, in contrast only one Caucasian vegetarian subject had two children (Table 3:4). The high nulliparity (94%) in Caucasian vegetarians could be due to a greater proportion of single women in the group and also that the mean age of Caucasian vegetarians was slightly lower than the other groups. The average household size was calculated by including all adults and children under the age of 16 years. Single women sharing a flat, but not cohabiting, were counted as 1 person per household. The average household size in the UK has been reported to be 2.48 in 1988 (Central Statistical Office, 1990) and the average sizes of Caucasian omnivores and Indian vegetarian households were significantly greater than

that of Caucasian vegetarians. The household size of 4.4 in Indians in this study corresponds to the national figure of 4 reported by the Labour Force Survey (1987) for Indians living in the UK.

TABLE 3:4

Number of children and mean household size with percentages in parentheses

	CO	IV	CV
<i>Nulliparous</i>	5 (20.8)	4 (16.7)	17 (94.4)
<i>1 Child</i>	5 (20.8)	5 (20.8)	0
<i>2 Children</i>	7 (29.2)	11 (45.8)	1 (5.6)
<i>3 or more Children</i>	7 (29.2)	4 (16.7)	0
<i>Mean Interval (yrs) since recent childbirth (SEM)</i>	6.3 (1.08)	8.5 (1.15)	2.0
<i>Mean Household Size (SEM)</i>	3.3 (0.23)	4.4 (0.29)	1.7 (0.18)

3:2 Socio-Economic Status

3:2:1 Education and Employment

The level of education often influences socio-economic status and general awareness of health and other issues. According to the Labour Force Survey (1988), approximately 70% of women aged 25 - 39 years had some qualification, either from University or other Technical Institutions. The level of education in Caucasian vegetarians was higher, 72% were graduates of a University or a Polytechnic (Table 3:5). Seven Indians also had been to University in India. The higher qualifications of the Caucasian vegetarians was reflected in the type of occupation they pursued: the majority were professionals.

TABLE 3:5

Educational establishment last attended (number and percentage in parentheses)

Educational Establishment	CO	IV	CV
<i>Elementary School</i>	0	2 (8.3)	0
<i>Secondary School</i>	7 (29.2)	10 (41.7)	1 (5.6)
<i>Technical Institute</i>	14 (58.3)	5 (20.8)	4 (22.2)
<i>University/Polytechnic</i>	3 (12.5)	7 (29.2)	13 (72.2)

Caucasian vegetarians were predominantly professional and none was self-employed (Table 3:6). One Indian subject was self-employed, managing a small supermarket; three Indians were professionals (teacher, radiographer, and social worker) and the others were mostly manual and clerical workers.

TABLE 3:6

Current occupation of the subjects (number and percentage in parentheses)

Occupation of Subject	CO	IV	CV
<i>Housewife</i>	3 (12.5)	3 (12.5)	0
<i>Domestic</i>	0	1 (4.2)	0
<i>Manual</i>	2 (8.3)	6 (25.0)	0
<i>Clerical</i>	10 (41.7)	7 (29.2)	3 (16.7)
<i>Skilled</i>	2 (8.3)	3 (12.5)	1 (5.6)
<i>Professional</i>	7 (29.2)	3 (12.5)	14 (77.8)
<i>Self-employed</i>	0	1 (4.2)	0

3:2:2 Home and Car Ownership

Home ownership in the Indian subjects was more popular than in the other groups. However, in both groups of Caucasians house or flat ownership was similar, with 50% of Caucasian vegetarians being flat owners (Table 3:7). Council tenancy was also higher in Caucasian omnivores and was similar in Indian and Caucasian vegetarians. A high proportion of Indians (87.5%) were car owners compared to other groups. Based on the above criteria, the Indian subjects appear to enjoy better socio-economic status, but other factors need to be considered. Home ownership is much coveted in this group nationally and also the extended family system may make this necessary. Several Indian households included other relatives, such as parents-in-law and both married and unmarried siblings.

TABLE 3:7

Home owners and car owners among the subjects (number and percentage in parentheses)

Ownership	CO	IV	CV
<i>House Owners</i>	14 (58.3)	19 (79.2)	3 (16.7)
<i>Flat Owners</i>	2 (8.3)	1 (4.2)	9 (50.0)
<i>Private Tenants</i>	2 (8.3)	1 (4.2)	3 (16.7)
<i>Council Tenants</i>	6 (25.0)	3 (12.5)	3 (16.7)
<i>Car Owners</i>	15 (62.5)	21 (87.5)	6 (33.3)

3:3 Health**3:3:1 Smoking Habits and Alcohol Consumption**

The smoking habits of the groups varied considerably, but a higher proportion of Caucasian omnivores were current and past smokers (Table 3:8). Alcohol consumption, as expected, was not popular with the Indians, although there is no religious dictum against its use. However, as Hindus believe alcohol to be a pollutant which challenges the value of self-control, abstinence from alcohol is practised widely, especially by women. Similar beliefs may be responsible for the low frequency of smoking found among the Indian subjects.

TABLE 3.8

Smoking habits and alcohol consumption (number and percentage in parentheses)

	CO	IV	CV
<u>Smoking Habits</u>			
<i>Non-Smokers</i>	10 (41.7)	23 (95.8)	14 (77.8)
<i>Current Smokers</i>	9 (37.5)	1 (4.2)	1 (5.6)
<i>Past Smokers</i>	5 (20.8)	0	3 (16.7)
<u>Alcohol Consumption</u>			
<i>Most Days</i>	1 (4.2)	0	2 (11.1)
<i>3-4 Times/Week</i>	5 (20.8)	0	3 (16.7)
<i>1-2 Times/Week</i>	11 (45.8)	2 (8.3)	9 (50.0)
<i>1-2 Times/Month</i>	5 (20.8)	2 (8.3)	3 (16.7)
<i>1-2 Times in 6 Months</i>	1 (4.2)	2 (8.3)	0
<i>1-2 Times/Year</i>	0	2 (8.3)	0
<i>Abstainers</i>	1 (4.2)	16 (66.5)	1 (5.6)

3:3:2 Use of Nutrient Supplements

Caucasian vegetarians were most likely to use nutrient supplements (Table 3:9). Two subjects each from both Caucasian groups were using γ -linolenic acid + Vitamin B₆ supplement and the reason given was for the relief of pre-menstrual symptoms. Two Indians were actually prescribed supplements of multivitamins, iron and calcium.

TABLE 3.9

Subjects taking nutrient supplements (numbers and percentages in parentheses)

Supplement Taken	CO	IV	CV
<i>Multivitamin + Iron</i>	2 (8.3)	4 (16.7)	4 (22.2)
<i>γ-Linolenic Acid + Vitamin B₆</i>	2 (8.3)	0	2 (8.3)
<i>Calcium</i>	0	1 (4.2)	0
Total using Supplements	4 (16.7)	5 (20.8)	6 (33.3)

3:3:3 Cervical Cytology

Subjects were questioned about the frequency with which they undertook cervical smear tests, in order to assess their awareness of health and importance of cytology in preventing cervical cancer. Indian women appear to be less inclined to take up smear tests compared to Caucasians. There were no abnormal smears reported in the Indians, but this could be due to the low uptake of cytology (Table 3:10).

TABLE 3:10

Frequency of cervical smear tests (number and percentage in parentheses)

	CO	IV	CV
<i>Never</i>	2 (8.3)	10 (41.7)	2 (11.1)
<i>Six Monthly</i>	3 (12.5)	0	1 (5.6)
<i>One Yearly</i>	1 (4.2)	0	2 (11.1)
<i>Two Yearly</i>	5 (20.8)	6 (25.0)	4 (22.2)
<i>Three Yearly</i>	11 (45.8)	5 (20.8)	9 (50.0)
<i>Five Yearly</i>	2 (8.3)	3 (12.5)	0
<i>Total No. of Subjects having Regular Tests</i>	<i>33 (91.7)</i>	<i>14 (58.3)</i>	<i>16 (88.9)</i>
<i>Number of Abnormal Smears</i>	3 (12.5)	0	2 (11.1)

3:3:4 History of Disease in the Past

All subjects who participated in the study were healthy and did not suffer from any illness through the duration of the study (Table 3:11). Four Indian subjects had suffered from viral hepatitis which conforms with the high mortality from liver cancer observed in Asians in the UK. Two omnivores and two Caucasian vegetarians had suffered from cancer of the cervix. Both omnivorous subjects had undergone hysterectomy, while the other Caucasian vegetarians were given non-surgical laser treatment. They were all free of any known carcinoma at the time of the study.

TABLE 3:11

Frequency of past diseases

	CO (n=24)	IV (n=24)	CV (n=18)
<i>Asthma</i>	1	1	1
<i>Viral Hepatitis</i>	1	4	0
<i>Cervical Intraepithelial Neoplasia</i>	2	0	2
<i>Cholecystectomy</i>	0	1	0

3:3:5 Family History of Disease

Frequency of cancer in the family was higher in the Caucasians compared to the Indians (Table 3:12). Incidence of cardiovascular disease was also higher in the omnivores, which is contrary to the high mortality from CHD seen in Asians in the UK. However, it must be noted that the parents of the Indian subjects were younger compared to Caucasians.

TABLE 3:12

Subjects with history of disease suffered by either parent (number and percentage in parentheses)

	CO	IV	CV
<i>Cardiovascular Disease</i>	6 (25.0)	3 (12.5)	1 (5.6)
<i>Diabetes</i>	0	1 (5.6)	1 (5.6)
<i>Cancer</i>	3 (12.5)	1 (4.2)	2 (11.1)
<i>Tuberculosis</i>	0	2 (8.3)	0

3:4 Blood Pressure

Systolic and Diastolic pressures were lower in the Indian women compared to the Caucasians, but the difference was not statistically significant. Blood pressure measurements were also lower in all subjects compared to British Women in the two age groups, except in Caucasian vegetarians, where mean diastolic pressure was higher than that of the younger age groups (25 - 34 years) but lower than that found in the older group (35 - 49 years). However, there was no significant correlation between blood pressure measurements and age or weight in the subjects.

TABLE 3:13

Diastolic and systolic blood pressure in the subjects

	British Women*		CO		IV		CV	
	25-34y	35-49y	Mean	SEM	Mean	SEM	Mean	SEM
<i>Systolic (mmHg)</i>	111	118	102	1.8	100.9	1.60	102.4	2.58
<i>Diastolic (mmHg)</i>	69	74	67.6	1.81	67.0	1.63	71.9	1.82

* *Source: Gregory et al. (1990).*

3:5 Anthropometric Characteristics

It was considered necessary to make anthropometric measurements of weight, height and skinfolds, as one of the aims of the study was to relate dietary intakes to physiological measurements. Body measurements themselves provide health indicators which can be compared over time for different groups of the population. It was also considered most important to have some measure of fatness in view of the established relationship between obesity and increased morbidity and mortality. Anthropometric measurements of the subjects are given in Table 3:4. Caucasian subjects, both omnivores and vegetarians, were taller than the Indians and similar in height to women in the general population. Body weights were greatest in the Caucasian omnivores and lowest in the Caucasian vegetarians. The Indian women were heavier for their height than either

Caucasian vegetarians or the Caucasian omnivores and this is reflected in the BMI values. The standard weight for height after allowing for frame size was also higher in the Indians, although the differences were not statistically significant.

TABLE 3:14

Anthropometric measurements of the subjects (mean \pm SEM)

	British Women*		Adult Women* London & SE	CO (n=24)	IV (n=24)	CV (n=18)
	25-34 y	35-49 y				
<i>Weight (kg)</i>	63.2 (0.87)	65.0 (0.61)	63.7 (0.64)	64.6 (2.41)	61.4 (2.67)	59.4 (2.60)
<i>Height (cms)</i>	162.6 (0.39)	161.9 (0.30)	162.7 (0.64)	163.6 ^a (1.57)	155.9 ^b (1.14)	160.9 ^a (1.94)
<i>BMI (wt/ht²)</i>	23.9 (0.33)	24.8 (0.21)	24.2 (0.24)	24.1 (0.70)	25.1 (0.98)	22.5 (1.00)
<i>% Wt/Ht</i>				116.2 (3.15)	121.0 (4.82)	109.0 (4.83)
<i>Biceps (mm)</i>				10.7 ^a (0.83)	9.9 ^a (0.68)	7.3 ^b (0.94)
<i>Triceps (mm)</i>				19.8 ^a (1.25)	22.0 ^a (0.91)	14.0 ^b (1.11)
<i>Subscapular (mm)</i>				16.4 ^a (1.20)	21.0 ^b (1.21)	13.0 ^a (1.86)
<i>Suprailiac (mm)</i>				16.3 ^a (1.56)	25.2 ^b (1.94)	13.6 ^a (2.19)
<i>Sum skinfolds (mm)</i>				63.2 ^a (3.90)	78.2 ^b (3.92)	47.9 ^a (5.38)
<i>Waist (cm)</i>				72.8 (1.79)	76.3 (2.35)	69.8 (2.82)
<i>Hip (cm)</i>				95.6 (2.21)	98.8 ^a (2.69)	90.9 ^b (2.46)
<i>Waist/Hip Ratio</i>				0.76 (0.007)	0.77 (0.014)	0.77 (0.015)
<i>Body Fat (%)</i>	29.5 (0.31)	31.5 (0.19)	30.3 (0.25)	30.7 ^a (0.96)	34.1 ^b (0.72)	26.2 ^a (1.32)

^{a,b,c}: Values not sharing similar superscripts are significantly different from each other

* Source: Gregory *et al.* (1990).

Body fat as a percentage of total weight was significantly greater in Indian compared to Caucasian groups and also to the average percentage of body fat in British women. Caucasian vegetarians differed from the general British women in that they were lighter in weight and also had lower amounts of body fat. Indian women as a group showed a different pattern of distribution of body fat compared to Caucasians. Centripetal fat measurements of subscapular and suprailiac skinfolds and hip circumference were all significantly raised in the Indians, with the exception of waist/hip ratio. This indicates that Indian women have a tendency for central obesity, known to be associated with hyperinsulinism and insulin resistance; the underlying features of diabetes and also coronary heart disease which are prevalent in excess in this group. A significant proportion of Indian women had waist/hip ratio greater than 0.8, which is regarded as a risk factor for diabetes and coronary heart disease (Table 3:15).

TABLE 3:15

Number of subjects (%) with BMI, waist/hip ratio and standard weight for height outside the normal ranges

	CO (n=24)	IV (n=24)	CV (n=18)
<i>BMI</i>			
< 20	0	0	5 (27.8)
> 25	5 (20.8)	10 (41.7)	3 (16.7)
<i>Standard Weight for Height</i>			
< 90%	0	0	2 (11.1)
> 120%	7 (29.2)	11 (45.8)	3 (16.7)
<i>Waist/Hip Ratio</i>			
> 0.80	3 (12.5)	10 (41.7)	3* (16.7)

* $p < 0.01$ (Chi-square).

3:6 Summary

1. The age of the subjects ranged from 25-40 years. Caucasian omnivores and Indian vegetarians were well matched for age, but the Caucasian vegetarians were slightly younger.
2. All the Indian subjects who participated in the study were born outside the UK
3. A majority of the Caucasian omnivores and Indians were married with children. Only one-third of the Caucasian vegetarians were married and one subject had children.
4. Approximately 75% of the Caucasian vegetarians were graduates and held professional qualifications, compared to 30% of omnivores and 13% of Indians.
5. All the Indian subjects were non-smoking except one, compared to 78% of Caucasian vegetarians. A high proportion of Indian subjects also abstained from alcohol.
6. The Indian subjects also had higher BMI, significantly greater sum of skinfolds and body fat compared to Caucasians.

CHAPTER 4

DIETARY CHARACTERISTICS

4:1 Food Consumption

Dietary records of the subjects were analyzed to obtain details of food consumption. Table 4:1 gives detailed quantities of the various foods consumed by the Caucasian omnivores, Indian and Caucasian vegetarians. Among cereal products bread was consumed by all subjects, but leavened bread was not so popular with the Indians who preferred unleavened bread - chapatis prepared at home. Breakfast cereals were consumed by 72.2% of the Caucasian vegetarians compared to 40% of omnivores and 52% of Indian women. The total amount of cereals consumed were similar in the vegetarian groups and higher than in the omnivores, although there was no statistically significant difference. The vegetarian groups also consumed higher amounts of pulses compared to the Caucasian omnivores. The quantity of pulses consumed by the Indians was lower than that consumed by the Caucasian vegetarians. Pulses in Indian cuisine are usually prepared as dhals, which are often dilute and of thin consistency, whereas the Caucasian vegetarians may prefer to cook pulses as roasts or thick casseroles.

Caucasian omnivores consumed higher amounts of total milk products and also fluid milk. Cheese was not popular with the Indians, and Caucasian vegetarians consumed the greatest amount of cheese compared to other groups. Cheese may have been widely used to compensate for the absence of meat or fish in the diet by the Caucasian vegetarians but not by the Indians, as few Indian dishes incorporate cheese and usually in the form of cottage cheese. Yoghurt was eaten by 81% of the Indians and 61% of Caucasian vegetarians compared to only 31.8% of omnivores. Egg consumption was the highest in the omnivorous group, but composite dishes made of eggs and cheese were equally popular with all groups. Use of spreading fats such as butter or margarines was similar in both Caucasian groups and considerably lower in the Indians and could be related to lower consumption of leavened bread in this group. Small quantities of ghee were used to spread on chapatis by 95% of the Indians.

Poultry was the most popular meat consumed by the omnivores, followed by canned meats such as corned beef and cooked meat dishes, usually made out of beef. Lamb was eaten by 27% of the omnivores and only 9% consumed liver during the seven day period of dietary assessment. White fish was more popular, compared to fatty fish and only 18% consumed shellfish, mostly prawns and shrimps. Fish products such as fish fingers were eaten by 13% of the omnivores.

Vegetable consumption was the lowest in the Indians and this may be due to the method of cooking curries which tend to be dilute. Consumption of potatoes was higher in the omnivores, mainly in the form of mashed potatoes or chips, whereas Indians tended to use potatoes in their curries. All kinds of fruits were consumed in higher amounts by the vegetarians, especially citrus fruits. Soft fruits such as berries were not so popular with the Indians. Fifty percent of Caucasian vegetarians consumed dried fruits. Nuts were also popular with the vegetarians. A majority of Indian women fasted one day per week which entailed eating only nuts, fruits and milk.

Seventy-one percent of the Indian women added sugar to tea or coffee compared to 50% of Caucasian omnivores and only 33% of Caucasian vegetarians. However, consumption of preserves such as jams and confectionery was lower in the Indians. Caucasian omnivores consumed higher amounts of hot beverages such as tea and coffee compared to other groups. Soft drinks, mainly fruit juices, were popular with the Caucasian vegetarians. Alcohol consumption was also higher in Caucasians as a majority of Indian women were teetotalers. Both vegetarian groups popularly prepared special recipes at home and therefore had a significantly higher consumption of miscellaneous foods. Total quantity of food eaten in a day was greater in the Caucasian vegetarians.

TABLE 4:1 Mean weight of foods consumed (g/day)

Foods	CO			IV			CV		
	Mean	Median	% who ate	Mean	Median	% who ate	Mean	Median	% who ate
<i>Grains + Flours + Starches</i>	39 ^a	40	81.8	78 ^b	72	100	66 ^c	48	100
<i>Bread</i>	88 ^a	93	100	107	103	100	121 ^b	116	100
<i>Breakfast Cereals</i>	15	0	40.9	12	3	52.4	20	15	72.2
<i>Cakes</i>	24	15	90.9	12	10	76.2	17	0	66.7
<i>Buns & Pastries</i>	10	3	59.1	9	4	57.1	9	0	44.4
<i>Puddings</i>	25	13	68.2	25	20	76.2	15	11	55.6
<i>Total Cereals</i>	200	205	-	242	209	-	248	238	-
<i>Pulses</i>	34 ^a	31	86.4	93 ^b	69	100	111 ^b	76	94.4
<i>Milk</i>	232	180	95.5	166	161	90.5	148	114	88.9
<i>Cream</i>	2	0	18.2	1	0	9.5	0.3	0.3	11.1
<i>Cheese</i>	15 ^a	11	72.7	12 ^b	0	38.1	29 ^a	32	77.8
<i>Yoghurt</i>	11	0	31.8	35	17	81.0	29	12	61.1
<i>Total Milk Products</i>	260	216		213	187		205	175	
<i>Eggs</i>	17 ^a	11	63.6	2 ^b	0	4.8	9	0	38.9
<i>Egg & Cheese Dishes</i>	22	0	36.4	20	6	52.4	23	9	44.4
<i>Total Egg Products</i>	39	27	-	22	16	-	32	30	-

TABLE 4:1 Mean weight of foods consumed (g/day)

continued

Foods	CO			IV			CV		
	Mean	Median	% who ate	Mean	Median	% who ate	Mean	Median	% who ate
Spreading Fats	15	15	100	6	6	95.2	15	17	94.4
Bacon	8	3	59.1	-	-	-	-	-	-
Beef	16	15	54.5	-	-	-	-	-	-
Lamb	7	0	27.3	-	-	-	-	-	-
Pork	14	11	54.5	-	-	-	-	-	-
Poultry	21	19	81.8	-	-	-	-	-	-
Heart	1	0	4.5	-	-	-	-	-	-
Liver	2	0	9.0	-	-	-	-	-	-
Canned Meats	15	13	72.7	-	-	-	-	-	-
Offal Products	1	0	9.0	-	-	-	-	-	-
Sausages	4	0	40.9	-	-	-	-	-	-
Meat Products	7	0	45.4	-	-	-	-	-	-
Meat & Pastry Products	2	0	9.0	-	-	-	-	-	-
Cooked Meat Dishes	50	42	72.7	-	-	-	-	-	-
Total Meat Products	148	155	-	-	-	-	-	-	-

TABLE 4:1 Mean weight of foods consumed (g/day)

continued

Foods	CO			IV			CV		
	Mean	Median	% who ate	Mean	Median	% who ate	Mean	Median	% who ate
<i>White Fish</i>	11	0	45.4	-	-	-	-	-	-
<i>Fatty Fish</i>	7	0	31.8	-	-	-	-	-	-
<i>Shell Fish</i>	2	0	18.2	-	-	-	-	-	-
<i>Fish Products</i>	2	1	13.6	-	-	-	-	-	-
Total Fish Products	22	20	-	-	-	-	-	-	-
<i>Vegetables</i>	50 ^a	48	90.9	88 ^b	88	100	102 ^b	89	100
<i>Potato</i>	106 ^a	94	100	33	20 ^b	76.2	59 ^b	46	94.4
<i>Root Vegetables</i>	17	5	54.5	6	0	52.4	26	11	55.6
<i>Leafy Vegetables</i>	39	32	100	27	19	76.2	45	43	94.4
Total All Vegetables	210	195	-	154 ^a	146	-	232 ^b	202	-
<i>Fruit</i>	31 ^a	23	63.6	88 ^b	64	76.2	54	31	72.2
<i>Hard Fruit</i>	15	3	50.0	43	36	71.4	96	15	55.6
<i>Soft Fruit</i>	8	0	36.4	3	0	19.1	7	0	38.9
<i>Citrus Fruit</i>	26	0	31.8	26	11	52.4	75	21	77.8
<i>Dried Fruit</i>	0.3 ^a	0	9.1	0.3 ^a	0	4.8	10 ^b	2	50.0
<i>Nuts</i>	4	0	40.9	10	3	61.9	12	7	72.2
Total Fruit + Nuts	84 ^a	49	-	171	102	-	254 ^b	125	-

TABLE 4:1 Mean weight of foods consumed (g/day)

continued

Foods	CO			IV			CV		
	Mean	Median	% who ate	Mean	Median	% who ate	Mean	Median	% who ate
<i>Sugars</i>	13	1	50.0	9	5	71.4	2	0	33.3
<i>Preserves</i>	5	0	36.4	1	0	4.8	5	0	50.0
<i>Confectionery</i>	7	3	68.2	3 ^a	0	23.8	12 ^b	8	66.7
Total Sugars, Preserves & Confectionery	25	13		13	6		20	13	
Hot Beverages	880 ^a	812	100	355 ^b	319	95.2	698 ^a	663	100
Soft Drinks	85 ^c	60	77.3	83 ^a	54	85.7	155 ^b	114	94.4
<i>Beers</i>	83	0	36.4	2	0	4.8	97	0	44.4
<i>Cider</i>	3 ^a	0	4.5	0	0	0	19 ^b	0	22.2
<i>Wines, Liqueurs & Spirits</i>	54	6	54.5	3	0	19.1	83 ^a	81	72.2
Total Alcoholic Beverages	140 ^a	53		5 ^b	0		200 ^a	133	
<i>Sauces</i>	18 ^a	14	95.5	3 ^b	1	52.4	12 ^c	7	77.8
<i>Soups</i>	12 ^a	0	18.2	10 ^a	0	33.3	57 ^b	29	61.1
<i>Miscellaneous (made-up dishes)</i>	12 ^a	0	27.3	172 ^b	75	90.5	139 ^b	74	94.4
Total Weight of Food Consumed	2182 ^a	2251		1541 ^b	1406		2389 ^a	2358	

^{a, b, c}: Values with unlike superscript letters are significantly different ($p < 0.05$)

4:2 Nutrient Intakes

The dietary intakes of macronutrients are given in Table 4:2.

4:2.1. Energy

Energy intakes were significantly lower in the Indian women (Table 4:2). Although they were smaller in stature, their body mass index (BMI) was greater than the other groups and lower energy intakes did not reflect in lower BMI. Total energy intake was lower in Indian women compared to the average intakes of British women, while intakes in the Caucasians were higher.

The coefficient of variation in daily individual intakes was slightly greater in the Indians, although the difference was not statistically significant. Indian women fasted at least one day per week, but this did not reflect in a significant difference in day-to-day variation in energy intakes within the groups. Unlike the Muslims, Hindu religious fasts entail eating fruits, milk, nuts etc. all of which contributed reasonable amounts of energy on those days. The energy density of the diet (i.e. energy provided per gram of food) varied between the groups. The diet of the Indian women was significantly more energy dense compared to the Caucasians. However, the diet of Caucasian vegetarians had the lowest energy density. The energy intakes in all groups were lower than the estimated average requirement of 1,940 kcal/d (COMA, 1991).

Table 4:4 shows the contribution of energy from different food groups. The bulk of the energy was provided by cereals in all groups, but in the Indian diet cereals contributed 39% of the total intake compared to 29% and 33% in the omnivorous and Caucasian vegetarian diets. Meat and fish products provided approximately 19% of the total energy in the omnivorous diet. Pulses made a more significant contribution to the total energy intake in the Indian diet compared to other diets. The proportion of energy contributed by beverages was higher in the Caucasian vegetarians and this was in agreement with the higher intakes of alcohol and fruit juices in this group. Miscellaneous foods also provided higher amounts of energy in the Caucasian vegetarian diet and may be due to the inclusion of home-made dishes, which were very popular in this group.

TABLE 4:2 Daily intakes of energy, protein, carbohydrate, fat, alcohol and fibre

Nutrients	CO (n=22)			IV (n=21)			CV (n=18)			British women 25-39 y
	Mean	Median	SE	Mean	Median	SE	Mean	Median	SE	
Energy (kcal)	1778 ^a	1835	76.6	1446 ^b	1427	70.5	1827 ^b	1868	88.7	1670
Protein (g)	70.3 ^a	66.1	4.13	42.4 ^b	42.1	2.41	54.1 ^c	53.6	2.79	59.5
% energy from Protein	15.8 ^a	15.1	0.58	11.7 ^b	11.2	0.42	11.9 ^b	12.0	0.34	14.6
Protein/1000 kals	39.5 ^a	37.8	1.45	29.3 ^b	27.9	1.06	29.8 ^b	30.0	0.84	35.6
Carbohydrate (g)	191 ^a	187	10.4	195 ^a	187	10.4	236 ^b	227	17.5	192
% energy from CHO	43 ^a	41.9	1.42	54.1 ^b	54.3	1.18	51.2 ^b	50.2	2.33	43
Carbohydrate/1000 kals	107 ^a	105	3.6	135 ^b	136	3.0	128 ^b	125	5.8	115
Lactose (mg)	11.8	9.2	2.08	9.4	8.5	1.63	8.9	7.9	1.45	-
Sugar (g)	75.2	69.6	7.41	59.6 ^b	58.8	5.55	91.1 ^b	85.0	11.57	85
Sugar/1000 kals	41.9	36.2	3.51	40.8	43.4	2.78	49.0	43.2	5.43	50.1
Starch (g)	92.5 ^a	93.0	4.99	92.5 ^a	87.6	5.67	119.5 ^b	110.9	8.80	106
Starch/1000 kals	53 ^a	52	2.2	64 ^b	63	2.4	65 ^b	66	3.2	63.8
Fat (g)	80.0 ^a	82.0	4.25	60.5 ^b	56.2	3.32	71.3	75.4	4.81	73.5
% energy from Fat	40.3 ^b	41.1	1.14	37.7	37.7	1.05	35.1 ^a	35.5	1.72	39.2
Fat/1000 kals	44.8 ^a	45.7	1.3	41.9	41.9	1.2	39 ^b	39.4	1.9	43.8
Alcohol (g)	8.4 ^a	5.5	1.94	0.6 ^b	0	0.29	11.6 ^c	10.15	2.36	7.7 10.8
% energy from Alcohol	3.4 ^a	2.5	0.78	0.3 ^b	0	0.13	4.9 ^a	3.3	1.01	3.1 4.3
Alcohol/1000 kals	4.8 ^a	3.6	1.11	0.36	0	0.18	6.9 ^a	4.8	0.68	-
Fibre (g)	16.6 ^a	15.6	0.97	16.2 ^a	15.6	1.17	29.3 ^b	29.1	2.57	18.2
Fibre/1000 kals	9.4 ^a	9.6	0.37	11.4 ^a	11.6	0.73	15.9 ^b	15.3	1.03	11.1

Source: * Gregory *et al.* (1990)

** Values in alcohol consumers only

^{a, b, c}: Values with unlike superscript letters are significantly different ($p < 0.05$)

TABLE 4:3

Coefficient of variation in energy intake and the energy density of the diets (mean \pm SEM)

	Caucasian Omnivores	Indian Vegetarians	Caucasian Vegetarians
<i>Mean coefficient of variation in energy intakes during 7-day assessment</i>			
<i>Total Energy</i>	21% (1.9)	24% (2.0)	22% (2.4)
<i>Food Energy</i>	20% (1.8)	24% (1.9)	23% (2.2)
<i>Energy density of the diet (kcal/g of food)</i>	0.85 ^a (0.040)	0.98 ^b (0.048)	0.79 ^a (0.042)

^{a,b} Values sharing unlike superscript letters are statistically significant from each other.

4:2.2. Protein

Protein intakes of the groups varied significantly (Table 4:2). Caucasian omnivores had the highest intake of protein which contributed 15.8% of the total energy, which was significantly greater than the proportion of energy derived from protein in vegetarian groups. The amount of protein per 1000 kcals and the proportion of energy from protein were both similar in the vegetarian groups, but considerably lower when compared to the average for British women. In contrast, protein intake in the Caucasian omnivores was higher than in the British women. Although the vegetarians had lower protein intakes, intake of Caucasian vegetarians was greater than the UK RNI of 45 g/day and the intake of the Indians was lower. However, there was no difference between the vegetarian groups in their protein intakes per 1000 kcals.

Cereals (Table 4:5) formed the major source of protein in the vegetarians, and meat products contributed most of the protein in the omnivorous diet. This may raise the question of quality of protein in the vegetarian diet, but as protein from pulses and milk products formed 34% of total intake the quality is unlikely to be low, as proteins from different sources in the vegetarian diet complement each other, thus improving the overall protein quality of the diet.

TABLE 4:4 Energy contributed by different food groups

Food Groups	CO		IV		CV	
	kcal	% of total	kcal	% of total	kcal	% of total
<i>Cereals</i>	509	28.6	566	39.1	599	32.8
<i>Pulses</i>	19	1.1	95	6.6	81	4.4
<i>Milk Products</i>	216	12.2	173	12.0	187	10.2
<i>Egg Products</i>	82	4.6	49	3.4	81	4.4
<i>Fat Spreads</i>	102	5.7	47	3.3	107	5.9
<i>Meat Products</i>	297	16.7	-	-	-	-
<i>Fish Products</i>	33	1.9	-	-	-	-
<i>Vegetables</i>	211	11.9	205	14.2	210	11.5
<i>Fruits & Nuts</i>	61	3.4	108	7.5	186	10.2
<i>Sugars, Confectionery & Preserves</i>	95	5.3	52	3.6	80	4.4
<i>Beverages</i>	118	6.6	100	6.9	165	9.0
<i>Miscellaneous</i>	35	2.0	52	3.6	131	7.2
Totals	1778	100	1446	100	1827	100

TABLE 4:5 Protein contributed by different food groups

Food Groups	CO		IV		CV	
	g	% of total	g	% of total	g	% of total
<i>Cereals</i>	14.1	20.1	15.7	37.2	18.4	34.0
<i>Pulses</i>	1.6	2.3	4.3	10.2	5.2	9.6
<i>Milk Products</i>	11.9	16.9	10.2	24.2	12.2	22.6
<i>Egg Products</i>	4.2	6.0	2.1	5.0	3.4	6.3
<i>Meat Products</i>	26.2	37.3	-	-	-	-
<i>Fish Products</i>	4.3	6.1	-	-	-	-
<i>Vegetables</i>	3.9	5.5	3.4	8.1	4.4	8.1
<i>Fruits & Nuts</i>	1.5	2.1	2.2	5.2	3.8	7.0
<i>Sugars, Confectionery & Preserves</i>	0.5	0.7	0.2	0.5	0.8	1.5
<i>Beverages</i>	1.5	2.1	2.5	5.9	1.5	2.8
<i>Miscellaneous</i>	0.6	0.9	1.8	4.3	4.4	8.1
Totals	70.3	100	42.2	100	54.1	100

A considerably higher proportion of protein was from beverages in the Indian diet and was probably due to the different method of making tea. A majority of Indian subjects drank tea brewed in a mixture of at least equal quantities of milk and water, which increased their intake of milk protein.

4:2.3. Carbohydrate

Caucasian omnivores and Indian vegetarians had similar intakes of carbohydrate compared to the British women, but the proportion of energy from carbohydrate was higher in the Indians (Table 4:2). The amount of carbohydrate per 1000 kcals was also higher in the Indians. Energy contributed by carbohydrate was significantly greater in the vegetarians compared to the omnivores and also the British women.

Starch consumption is significantly greater in the Caucasian vegetarians and was related to higher consumption of bread and grains in this group. However, the amount of starch consumed per 1000 kcals is similar to the Indian vegetarians and British women and was significantly greater than in the Caucasian omnivores.

Sugar intake was significantly greater in the Caucasian vegetarians (Table 4:6). One-third of the total sugar intake in this group was from fruit and nuts, the intakes of which were significantly greater. Beverages also contributed considerably to the sugar intake of the Caucasian vegetarians. Fruits and nuts contributed most of the sugar in the diet of Indian vegetarians, but not in the omnivorous diets. However, sugar from confectionery, preserves and added sugar formed the major portion of sugars in the omnivorous diet.

4:2.4. Fat

Total fat intakes were higher in the Caucasian omnivores and so was the percentage of energy contributed by fat (40.3%), and this compared well with the mean values reported for British women. Indian vegetarians had the lowest fat intakes, but the intake per 1000 kcals and energy contributed by fat was higher than in the Caucasian vegetarians and only the difference between Caucasian vegetarians and omnivores achieved statistical significance (Table 4:2). Fat intakes in Caucasian vegetarians were in accordance with the COMA recommendation of 33% of energy from fat (COMA, 1991), but fell short of the WHO (1990) goal of 30% of energy from fat.

TABLE 4:6 Sugar contributed by different food groups

Food Groups	CO		IV		CV	
	g	% of total	g	% of total	g	% of total
<i>Cereals</i>	16.7	22.2	10.0	16.8	14.9	16.4
<i>Pulses</i>	0.8	1.1	0.6	1.0	1.2	1.3
<i>Milk Products</i>	12.7	16.9	10.3	17.3	10.5	11.5
<i>Meat Products</i>	0.4	0.5	-	-	-	-
<i>Vegetables</i>	3.3	4.4	1.6	2.7	4.6	5.0
<i>Fruits & Nuts</i>	7.9	10.5	15.7	26.3	28.4	31.2
<i>Sugars, Confectionery & Preserves</i>	20.7	27.5	11.7	19.6	13.5	14.8
<i>Beverages</i>	11.9	15.8	8.2	13.8	15.2	16.7
<i>Miscellaneous</i>	0.9	1.2	1.5	2.5	2.9	3.2
Totals	75.2	100	59.6	100	91.1	100

The COMA report also recommended limiting the energy intake from saturated fat to not more than 11% of the total energy, while the recommendation by WHO (1990) was 10%. Both groups of vegetarians met the COMA recommendation, but did not achieve the WHO recommendation of 10%. However, the median value of energy from saturated fat in the Caucasian vegetarians was 10% (Table 4:7).

Although the total fat intakes of omnivores in this study were slightly greater than the values reported for British women, their saturated fat intake was lower, whereas monounsaturated and polyunsaturated fat intakes (particularly n-6 fatty acids) were greater. Total monounsaturated fat intake was significantly lower in the Indians but there was no significant difference between the groups in the proportion of energy from monounsaturates. Intakes of n-6 polyunsaturated fatty acids were higher in both vegetarian groups compared to Caucasian omnivores and British women, but the intakes of n-3 fatty acids were significantly lower in the Indians. Intakes of individual polyunsaturated fatty acids are given in Table 6:2. Although both Indians and Caucasians were vegetarians and did not include any fish in their diet, the Caucasian vegetarians had a significantly higher intake of n-3 fatty acids which was in the form of α -linolenic acid, probably contributed by soya products which were popular in their diet.

Cereals, vegetables and milk products provided the majority of fat in the Indian diet; 21% from vegetables which are usually prepared as curries to which some fat is added (Table 4:8). Beverages also contributed 4% of total fat in the Indians which was mainly due to the greater quantities of milk in their tea or coffee. Meat and fish products contributed a quarter of the total fat in the omnivorous diet. A considerable contribution (14-16%) to the total fat was made by fat spreads in both Caucasian groups, whereas use of spreading fats was not popular with the Indians.

The ratio of n-6 : n-3 fatty acids was also significantly higher in the Indian diet, but the ratios in both groups of Caucasians were closer to the average values reported for British women. However, both the vegetarian diets had considerably higher P : S ratios compared to the omnivorous diet and to that of British women. As expected, cholesterol intakes were highest in the omnivores followed by the Caucasian vegetarians, where egg products may have contributed to their higher intakes compared to the Indians.

TABLE 4:7 **Quality of dietary fat**

Nutrients	CO (n=22)			IV (n=21)			CV (n=18)			British Women aged 25-34 y
	Mean	Median	SE	Mean	Median	SE	Mean	Median	SE	
Total Fat (g)	80.0 ^a	82.0	4.25	60.5 ^a	56.2	3.32	71.3	75.4	4.81	73.6
% Energy from Total Fat	40.3 ^a	41.1	1.14	37.7	37.7	1.05	35.1 ^a	35.5	1.72	39.4
Saturated Fatty Acids (g)	31.4 ^a	28.3	2.07	18.5 ^b	16.4	1.42	22.6 ^b	21.8	1.92	30.9
% of Saturated Fatty Acids	41.0 ^a	41.5	1.41	31.9 ^b	31.2	1.53	33.7 ^b	31.2	2.38	-
% Total Energy from Saturated Fat	15.7 ^a	15.0	0.57	11.4 ^b	11.5	0.60	11.4 ^b	10.1	1.01	16.4
Monounsaturated Fatty Acids (g)	31.9 ^a	32.7	1.99	23.8 ^b	22.2	1.33	29.4 ^a	30.0	2.34	22.6
% Monounsaturated Fatty Acids	41.4	42.6	1.19	42.0	41.0	1.63	43.0	44.6	1.64	-
% Total Energy from Monounsaturated Fatty Acids	16.1	16.6	0.73	15.1	14.9	0.74	14.3	14.6	0.82	12.0
Polyunsaturated n-6 Fatty Acids (g)	11.9	11.5	1.2	14.2	14.4	1.25	14.6	12.8	1.69	9.9
% Total Energy from n-6 Fatty Acids	6.39	6.14	0.656	7.60	7.71	0.672	7.84	6.88	0.908	5.32
n-3 Fatty Acids (g)	1.18	1.22	0.07	0.87 ^b	0.77	0.102	1.51	1.35 ^b	0.168	1.35
% Energy from n-3 Fatty Acids	0.63	0.65	0.039	0.47 ^b	0.41	0.548	0.81	0.72 ^b	0.090	0.74
Ratio of n-6:n-3 Fatty Acids	10.8 ^a	9.4	1.23	19.2 ^b	21.7	2.04	10.7 ^a	9.5	1.12	-
Ratio of Polyunsaturated to Saturated Fatty Acids	0.46 ^a	0.42	0.054	0.88 ^b	0.80	0.082	0.79 ^b	0.69	0.689	0.39
Cholesterol (mg)	199 ^a	200	22.7	34 ^b	19	12.3	104 ^b	82	20.5	264

^{a,b}: Values with unlike superscript letters are significantly different.

TABLE 4:8 Total fat contributed by different food groups

Food Groups	CO		IV		CV	
	g	% of total	g	% of total	g	% of total
<i>Cereals</i>	12.8	16.0	15.6	25.8	12.9	18.1
<i>Pulses</i>	0.3	0.4	4.1	6.8	2.4	3.4
<i>Milk Products</i>	13.4	16.8	10.0	16.5	11.0	15.4
<i>Egg Products</i>	5.5	6.9	2.7	4.5	5.4	7.6
<i>Fat Spreads</i>	11.4	14.3	5.3	8.8	11.8	16.5
<i>Meat Products</i>	19.0	23.8	-	-	-	-
<i>Fish Products</i>	1.6	2.0	-	-	-	-
<i>Vegetables</i>	9.1	11.4	12.8	21.2	11.5	16.1
<i>Fruits & Nuts</i>	2.4	3.0	3.7	6.1	6.2	8.7
<i>Sugars, Confectionery & Preserves</i>	1.7	2.1	0.7	1.2	2.6	3.7
<i>Beverages</i>	0.2	0.3	2.5	4.1	0.1	0.1
<i>Miscellaneous</i>	2.6	3.3	3.1	5.1	7.4	10.4
Totals	80.0	100	60.5	100	71.3	100

4:2.5. Alcohol

A majority of the Indian women in the study abstained from alcohol, and therefore the mean intakes were very low (Table 4:2). Alcohol consumption was higher in the Caucasian vegetarians compared to omnivores in this study, as well as mean intakes in British women. Wine drinking was more popular in Caucasian vegetarians and often accompanied the main meal of the day.

4:2.6. Fibre

The popular assumption that vegetarian diets are high in fibre could not be applied to the Indian vegetarians in this study. Total fibre intakes were similar in Caucasian omnivores and Indians, while they were considerably greater in the Caucasian vegetarians (Table 4:2). However, fibre intake per 1000 kcals was higher in Indians compared to Caucasian omnivores, but did not achieve statistical significance. Caucasian vegetarians as a group were highly motivated to healthy eating and consciously included considerable amounts of wholefoods in their diet, whereas the Indian vegetarian diet consisted of dehusked pulses, especially dhals, and chapati flour which is only about 70-80% wholemeal. However, cereals and pulses contributed a majority of fibre in the diets of all groups, but the vegetarian groups had significant quantities of fibre from fruits and nuts (Table 4.9).

TABLE 4:9 Dietary fibre contributed by different food groups

Food Groups	CO		IV		CV	
	g	% of total	g	% of total	g	% of total
<i>Cereals</i>	7.9	47.6	7.3	45.1	12.5	42.7
<i>Pulses</i>	2.4	14.5	1.4	8.6	3.2	10.9
<i>Egg Products</i>	0.3	1.8	0.3	1.9	0.2	0.7
<i>Meat Products</i>	0.8	4.8	-	-	-	-
<i>Vegetables</i>	3.5	21.1	2.7	16.7	5.9	20.1
<i>Fruits & Nuts</i>	1.6	9.6	3.2	19.8	6.1	20.8
<i>Miscellaneous</i>	0.1	0.6	1.3	8.0	1.4	4.8
Totals	16.6	100	16.2	100	29.3	100

4:2.7. Vitamin A

Mean intakes of vitamin A were all above the recommended daily amount of 750 μ g (Table 4:10). The vegetarians had lower intakes compared to the omnivores in this study, as well as the British women. Only 9% of omnivores ate liver during the week (Table 4:1).

4:2.8. Vitamin C

The recommended daily amount of vitamin C is currently 40 mg/day and the mean intakes of vitamin C in the Caucasian omnivores and Indians was considerably greater (Table 4:10). However, in the Caucasian vegetarians the intakes were three times higher, which was related to their higher intake of citrus fruits and fruit juice.

4:2:9 Vitamin D

Indian vegetarians had significantly lower vitamin D intakes compared to the Caucasians and also to mean intakes in the British women (Table 4:10). Sources of vitamin D are few and are most likely to be of animal origin. Most of the vitamin D intake of vegetarians was from fat spreads, but their use of was not popular with the Indians and therefore could have led to lower intakes (Table 4:11). Although there is no set recommended intake for vitamin D, in view of the fact that they are prone to rickets and osteomalacia, there is a clear need to augment their intakes.

4:2:10 Vitamin E

Vitamin E intakes were significantly greater in Caucasian vegetarians compared to Caucasian omnivores and Indians, but lower than the mean intakes of British women (Table 4:10). Caucasian vegetarians consumed larger amounts of wholegrain cereals and nuts which provided considerable amounts of vitamin E.

TABLE 4:10 Daily vitamin intakes excluding supplements

Nutrients	CO (n=22) Geometric Mean (95% Confidence Intervals)	IV (n=21) Geometric Mean (95% Confidence Intervals)	CV (n=18) Geometric Mean (95% Confidence Intervals)	British Women aged 25- 34 y						
Vitamin A (RE)	949 (671 - 1342)	715 (572 - 895)	946 (720 - 1242)	1234						
Per 1000 kCals	546 (400 - 744)	506 (409 - 626)	529 (419 - 666)	780						
	Mean	Median	SE	Mean	Median	SE				
Total Vitamin C (mg)	68.7 ^a	55.0	9.03	66.1 ^a	62.0	5.74	11.4 ^a	96.5	18.78	55.9
Per 1000 kCals	38.9 ^a	32.4	5.72	46.2	43.6	3.42	61.5 ^b	49.3	8.78	34.2
Total Vitamin D (µg)	2.03 ^a	1.75	0.299	0.66 ^b	0.30	0.148	1.59 ^a	1.25	0.249	2.3
Per 1000 kCals	1.18 ^a	0.99	0.189	0.44 ^b	0.22	0.090	0.86 ^b	0.81	0.123	1.42
Total Vitamin E (mg)	4.25 ^a	4.10	0.325	3.52 ^a	2.90	0.486	6.47 ^b	6.40	0.697	7.3
Per 1000 kCals	2.41 ^a	2.50	0.162	2.40 ^a	2.06	0.312	3.46 ^b	3.41	0.278	4.31

^{a,b}: Values with unlike superscripts are significantly different from each other.

TABLE 4:11 Vitamin D contributed by different food groups

Food Groups	CO		IV		CV	
	µg	% of total	µg	% of total	µg	% of total
<i>Cereals</i>	0.34	16.7	0.11	16.7	0.22	13.8
<i>Milk Products</i>	0.08	3.9	0.07	10.6	0.07	4.4
<i>Egg Products</i>	0.38	18.7	0.05	7.6	0.26	16.4
<i>Fat Spreads</i>	0.61	30.0	0.30	45.5	0.81	50.9
<i>Meat Products</i>	0.02	1.0	-	-	-	-
<i>Fish Products</i>	0.48	23.6	-	-	-	-
<i>Vegetables</i>	0.01	0.5	0.07	10.6	0.02	1.3
<i>Beverages</i>	0.04	2.0	0.02	3.0	0.12	7.5
<i>Miscellaneous</i>	0.07	3.5	0.04	6.1	0.09	5.7
Totals	2.03	100	0.66	100	1.59	100

4:2:11 The B Group of Vitamins

Thiamin, riboflavin and niacin intakes of the subjects were all according to the UK reference values (Table 4:12). However, when compared to FAO/WHO recommendations of 0.55 mg/1000 kcals for riboflavin and 6.6 mg/1000 kcals of niacin, the intakes in the Indians were adequate. Pyridoxine and biotin intakes were significantly lower in the Indians compared to both groups of Caucasians and pyridoxine intakes were below the UK reference value.

Vitamin B₁₂ intakes in the vegetarians were significantly lower than the omnivores, but the intake in Indians was half that of the Caucasian omnivores (Table 4:12). However, intakes in both vegetarian groups fell well below the U.S. recommended amounts of 2 µg/day. Vitamin B₁₂ intakes in Indians were also lower than the latest UK reference value of 1.2 µg/day. Approximately 72% of vitamin B₁₂ in the omnivorous diet was obtained from animal products, whereas about 75% of total vitamin B₁₂ intake in Indians was from milk products, but they are not particularly good sources of vitamin B₁₂ (Table 4:13). In contrast, in the Caucasian vegetarian diet, considerable amounts of vitamin B₁₂ were obtained from egg products, beverages - especially beer and miscellaneous foods which included made-up dishes as well as processed spreads such as Marmite, Vecon etc.

Total folate intakes (Table 4:12) were significantly lower in Caucasian omnivores and Indian vegetarians compared to Caucasian vegetarians and were below the UK reference value of 200 µg/day. Most of the folate in different diets was from cereals which are only fair sources of folate (Table 4:14). Consumption of good sources of folate, such as liver, was low in the omnivores and that of green leafy vegetables was low in the Indians. Only 9% of the omnivores consumed liver, while only 76% of Indians ate green leafy vegetables, compared to 90-100% of the Caucasians. It is also necessary to consider loss of folate during cooking by the Indians as they prefer cooked vegetables and rarely eat them raw. Fruits and nuts are also fairly good sources of folate and consumption of these was higher in the Caucasian vegetarians, which could account for their greater folate intakes.

TABLE 4:12 Daily mean intake of B vitamins excluding supplements

Nutrients	CO (n=22)			IV (n=21)			CV (n=18)			British Women aged 25-34 y
	Mean	Median	SE	Mean	Median	SE	Mean	Median	SE	
Total Thiamin (mg)	1.09 ^a	0.98	0.076	0.85 ^b	0.83	0.049	1.264 ^a	1.250	0.102	1.21
Per 1000 kCals	0.61	0.59	0.025	0.60 ^a	0.57	0.025	0.69 ^b	0.66	0.036	0.74
Total Riboflavin (mg)	1.58 ^a	1.50	0.168	1.09 ^b	1.05	0.079	1.33	1.26	0.094	1.5
Per 1000 kCals	0.86	0.76	0.064	0.77	0.75	0.055	0.73	0.71	0.039	0.91
Total Niacin (mg)	29.0 ^a	27.3	1.89	14.4 ^b	13.6	0.78	21.8 ^c	22.1	1.14	27.7
Per 1000 kCals	16.3 ^a	15.9	0.71	10.1 ^b	10.0	0.37	12.0 ^c	11.9	0.42	17.1
Total Vitamin B ₁₂ (µg)	5.52 ^a	3.69	1.25	0.87 ^b	0.87	0.127	1.52 ^b	1.56	0.157	4.5
Per 1000 kCals	2.98 ^a	2.12	0.607	0.60 ^b	0.51	0.093	0.85 ^b	0.88	0.082	2.9
Total Folate (µg)	170 ^a	163	13.9	142 ^a	140	10.2	262 ^b	234	34.1	206
Per 1000 kCals	95 ^a	93	5.8	100 ^a	93	7.0	139 ^b	124	12.3	126
Total Pyridoxine (mg)	1.19 ^a	1.05	0.112	0.75 ^b	0.60	0.065	1.29 ^a	1.25	0.134	1.54
Per 1000 kCals	0.66 ^a	0.63	0.038	0.53 ^b	0.44	0.048	0.69 ^a	0.69	0.052	0.94
Total Biotin (mg)	20.2 ^a	20.4	1.58	9.3 ^b	9.2	1.02	20.3 ^a	18.4	2.44	26.6
Per 1000 kCals	11.2 ^a	10.8	0.664	6.3 ^b	6.4	0.60	10.9 ^a	10.0	0.85	16.2

^{a,b,c}: Values with unlike superscripts are significantly different from each other.

TABLE 4:13 Vitamin B₁₂ contributed by different food groups

Food Groups	CO		IV		CV	
	µg	% of total	µg	% of total	µg	% of total
<i>Cereals</i>	0.17	3.1	0.12	13.8	0.05	3.3
<i>Pulses</i>	0.01	0.2	0	0	0.07	4.6
<i>Milk Products</i>	0.89	16.1	0.65	74.8	0.72	47.4
<i>Egg Products</i>	0.37	6.7	0.09	10.3	0.26	17.1
<i>Meat Products</i>	3.13	56.7	-	-	-	-
<i>Fish Products</i>	0.81	14.7	-	-	-	-
<i>Beverages</i>	0.13	2.4	0	0	0.13	8.6
<i>Miscellaneous</i>	0.01	0.1	0.01	1.2	0.29	19.1
Totals	5.52	100	0.87	100	1.52	100

TABLE 4:14 Folate contributed by different food groups

Food Groups	CO		IV		CV	
	µg	% of total	µg	% of total	µg	% of total
<i>Cereals</i>	51	30.0	49	34.5	70	26.7
<i>Pulses</i>	13	7.7	10	7.0	16	6.1
<i>Milk Products</i>	15	8.8	12	8.5	13	5.0
<i>Egg Products</i>	6	3.5	5	3.5	5	1.9
<i>Meat Products</i>	14	8.2	-	-	-	-
<i>Fish Products</i>	2	1.2	-	-	-	-
<i>Vegetables</i>	43	25.3	29	20.4	56	21.4
<i>Fruits & Nuts</i>	14	8.2	19	13.4	46	17.6
<i>Sugars, Confectionery & Preserves</i>	1	0.6	-	-	1	0.4
<i>Beverages</i>	8	4.7	5	3.5	13	5.0
<i>Miscellaneous</i>	3	1.8	13	9.2	44	16.8
Totals	170	100	142	100	262	100

4:2:12 Minerals

Calcium intakes in all groups were adequate and above the recommended amounts (Table 4:15). Most of the calcium in the diets was obtained from milk products, followed by cereals (Table 4:16). The doubts raised on the availability of calcium from vegetable sources could be dispelled by the higher proportion of calcium contributed by milk products which is known to be better absorbed and assimilated in the body.

Vegetable sources of iron contributed the majority of iron in the diets of both vegetarians and omnivores, but meat contributed 22% of the iron in the omnivorous diets (Tables 4:15 & 4:17). Average iron intakes in both omnivores and vegetarians were below the UK RNI of 14.8 mg/day but higher than the mean intakes of iron reported in British women. Since total iron intakes were low in all groups, it may be necessary to consider the efficacy of absorption of iron from vegetable sources due to the presence of inhibitors such as phytates, particularly in the vegetarians.

Copper and zinc intakes in pregnant Asian women have been reported to be low (Wharton *et al.*, 1984) and similarly significantly low intakes were found in this study. Both copper and zinc intakes were well below the UK RNIs (Table 4:15). Meat products were the main sources of copper and zinc in the omnivorous diet, while a majority of these was obtained from cereals, vegetables, fruits and nuts in the vegetarians (Tables 4:18 & 4:19). Caucasian vegetarians consumed higher amounts of vegetables and fruits which provided a fair amount of copper and zinc in the diet, but like other minerals the extent to which these are absorbed needs to be considered. Both copper and zinc are involved in vital enzyme systems and copper has been known to be implicated in the aetiology of nutritional anaemia, while deficiency of zinc has been attributed to growth faltering in populations consuming unleavened bread.

It was not easy to assess dietary intakes of sodium and potassium because of the difficulty in assessing the amount of added salt to food. Moreover, excretion of sodium and potassium is closely related to dietary intakes. Dietary assessment of sodium and potassium indicated that the Indians had significantly lower intakes of both but the urinary excretion of sodium was higher in the Indians compared to the Caucasian vegetarians (Table 4:20). This could mean that there was more added salt in the Indian diet, but in all groups the excretion of sodium was higher than that of dietary intake. In contrast, excretion of potassium was lower in all groups compared to the dietary intakes.

TABLE 4:15 Daily intake of minerals excluding supplements

Nutrients	CO (n=22)			IV (n=21)			CV (n=18)			British Women aged 25-34 y
	Mean	Median	SE	Mean	Median	SE	Mean	Median	SE	
Total Calcium (mg)	792	744	69.2	790	742	59.3	878	761	75.1	699
Per 1000 kCals	438 ^a	418	26.5	551 ^b	546	35.6	482	470	32.7	417
Total Iron (mg)	12.1	10.3	1.50	12.7	11.2	1.33	13.8	13.1	1.0	10.2
Per 1000 kCals	6.6 ^a	5.9	0.57	9.2 ^b	7.3	1.23	7.5	7.4	0.33	6.23
Total Copper (mg)	1.4 ^a	1.2	0.16	0.8 ^b	0.7	0.05	1.5 ^c	1.4	0.12	1.15
Per 1000 kCals	0.79 ^a	0.66	0.073	0.52 ^b	0.53	0.030	0.81 ^c	0.74	0.040	0.7
Total Zinc (mg)	8.9 ^a	8.6	6.4	4.2 ^b	4.4	0.31	7.4 ^c	7.5	0.51	8.2
Per 1000 kCals	5.0 ^a	5.0	0.23	2.9 ^b	3.0	0.17	4.1 ^c	4.0	0.18	4.98

^{a,b,c}: Values with unlike superscripts are significantly different from each other.

TABLE 4:16 Calcium contributed by different food groups

Food Groups	CO		IV		CV	
	mg	% of total	mg	% of total	mg	% of total
<i>Cereals</i>	163	20.6	143	18.1	154	17.5
<i>Pulses</i>	12	1.5	41	5.2	47	5.4
<i>Milk Products</i>	403	50.9	347	43.9	376	42.8
<i>Egg Products</i>	55	6.9	47	5.9	51	5.8
<i>Fat Spreads</i>	1	0.1	-	-	1	0.1
<i>Meat Products</i>	32	4.0	-	-	-	-
<i>Fish Products</i>	20	2.5	-	-	-	-
<i>Vegetables</i>	47	5.9	57	7.2	104	11.8
<i>Fruits & Nuts</i>	13	1.6	25	3.2	50	5.7
<i>Sugars, Confectionery & Preserves</i>	13	1.6	5	0.6	21	2.4
<i>Beverages</i>	26	3.3	90	11.4	29	3.3
<i>Miscellaneous</i>	7	0.9	35	4.4	45	5.1
Totals	792	100	790	100	878	100

TABLE 4:17 Iron contributed by different food groups

Food Groups	CO		IV		CV	
	mg	% of total	mg	% of total	mg	% of total
<i>Cereals</i>	5.3	43.8	5.9	46.5	5.4	39.1
<i>Pulses</i>	0.5	4.1	1.3	10.2	1.4	10.1
<i>Milk Products</i>	0.2	1.7	0.2	1.6	0.2	1.4
<i>Egg Products</i>	0.6	5.0	0.2	1.6	0.4	2.9
<i>Meat Products</i>	2.6	21.5	-	-	-	-
<i>Fish Products</i>	0.2	1.7	-	-	-	-
<i>Vegetables</i>	1.3	10.7	2.8	22.1	2.4	17.4
<i>Fruits & Nuts</i>	0.5	4.1	0.9	7.1	1.3	9.4
<i>Sugars, Confectionery & Preserves</i>	0.1	0.8	0.1	0.7	0.3	2.2
<i>Beverages</i>	0.7	5.8	0.4	3.2	1.5	10.9
<i>Miscellaneous</i>	0.1	0.8	0.9	7.1	1.0	7.2
Totals	12.1	100	12.7	100	13.8	100

TABLE 4:18 Copper contributed by different food groups

Food Groups	CO		IV		CV	
	mg	% of total	mg	% of total	mg	% of total
<i>Cereals</i>	0.33	23.2	0.30	40.0	0.50	33.6
<i>Pulses</i>	0.06	4.2	0.04	5.3	0.10	6.7
<i>Milk Products</i>	0.06	4.2	0.06	8.0	0.06	4.0
<i>Egg Products</i>	0.04	2.8	0.03	4.0	0.03	2.0
<i>Meat Products</i>	0.43	30.3	-	-	-	-
<i>Fish Products</i>	0.04	2.8	-	-	-	-
<i>Vegetables</i>	0.25	17.6	0.11	14.7	0.29	19.5
<i>Fruits & Nuts</i>	0.07	4.9	0.12	16.0	0.23	15.4
<i>Sugars, Confectionery & Preserves</i>	0.03	2.1	0.01	1.3	0.05	3.4
<i>Beverages</i>	0.10	7.0	0.03	4.0	0.12	8.1
<i>Miscellaneous</i>	0.01	0.7	0.05	6.7	0.11	7.4
Totals	1.42	100	0.75	100	1.49	100

TABLE 4:19 Zinc contributed by different food groups

Food Groups	CO		IV		CV	
	mg	% of total	mg	% of total	mg	% of total
<i>Cereals</i>	2.1	23.6	1.9	45.2	3.3	44.6
<i>Pulses</i>	0.2	2.2	0.1	2.4	0.3	4.1
<i>Milk Products</i>	1.4	15.7	1.2	28.6	1.4	18.9
<i>Egg Products</i>	0.5	5.6	0.3	7.1	0.4	5.4
<i>Meat Products</i>	3.5	39.3	-	-	-	-
<i>Fish Products</i>	0.2	2.2	-	-	-	-
<i>Vegetables</i>	0.5	5.6	0.2	4.8	0.6	8.1
<i>Fruits & Nuts</i>	0.2	2.2	0.3	7.1	0.6	8.1
<i>Beverages</i>	0.2	2.2	0.1	2.4	0.3	4.1
<i>Miscellaneous</i>	0.1	1.1	0.1	2.4	0.5	6.8
Totals	8.9	100	4.2	100	7.4	100

TABLE 4:20 Daily dietary intakes and urinary excretion of sodium and potassium

	CO (n=22)			IV (n=21)			CV (n=18)			British Women aged 25-34 y
	Mean	Median	SE	Mean	Median	SE	Mean	Median	SE	
Urine Volume (ml/24 hrs)	1611 ^a	1495	134	1166 ^b	1140	101	1469	1300	107	1345
Dietary Sodium (mg)	2345 ^a	2307	135	1856 ^b	1866	120	2387 ^a	2231	155	2372
Urinary Sodium (mg/24 hrs)	3092	3060	225	2979	3050	234	2517	2375	155	3013
Correlation Coefficient Dietary vs Urinary	0.40 (p=0.04)			- 0.16 (p=0.245)			0.07 (p=0.385)			0.26 (p<0.01)
Dietary Potassium (mg)	2750	2647	153	2327 ^a	2356	119	3229 ^b	3200	284	2324
Urinary Potassium (mg/24 hrs)	2384 ^a	2285	201	1901 ^b	1790	129	2676 ^a	2875	153	2418
Correlation Coefficient Dietary vs Urinary	0.53 (p=0.008)			- 0.11 (p=0.321)			0.008 (p=0.488)			0.36 (p<0.01)
Correlation Coefficients All Groups				Dietary vs Urinary Sodium - 0.048 (p=0.360) Dietary vs Urinary Potassium - 0.29 (p=0.012)						

^{a,b}: Values with unlike superscripts are significantly different from each other.

4:3 Summary

1. Nutrient intakes were assessed in pre-menopausal Caucasian omnivores, Indian and Caucasian vegetarians by seven-day weighed inventory.
2. Consumption of cereals, pulses and fruits was greater in the vegetarians. Omnivores consumed higher amounts of milk and milk products. Cheese was more popular with the Caucasian vegetarians, while the Indians preferred yoghurt. Eggs were also generally more popular with both the Caucasian omnivores and vegetarians. The Indian vegetarians had very low intakes of alcohol. Caucasian vegetarians preferred wine to other alcoholic drinks, unlike the omnivores. The total amount of food consumed per day was also greater in the Caucasians, the highest consumption being in the Caucasian vegetarians.
3. Energy intakes were significantly lower in the Indian vegetarians. The coefficient of variation in daily energy intakes was slightly greater in the Indians. Caucasian vegetarian diets had the lowest energy density and the bulk of the energy was provided by cereals in all groups. Protein intakes varied significantly between groups. The Caucasian omnivores had the highest intake of protein and the intakes were similar in both groups of vegetarians. Cereals were the major source of protein in the vegetarian diets and meat products contributed most of the protein in the omnivorous diet. Carbohydrate intakes were greater in the Caucasian vegetarians, but the proportion of energy from carbohydrates was greater in the Indians. Total fat, the proportion of energy from fat and saturated fat were greater in the Caucasian omnivores. The percentage of energy from saturated fat was similar in both vegetarian groups. The Caucasian vegetarians had a greater intake of n-3 fatty acids, probably contributed by soya products, in the diet. Cholesterol intakes were markedly lower in the Indians and intermediate in the Caucasian vegetarians. Fibre intakes were similar in Caucasian omnivores and Indian vegetarians and approximately half of the intakes in Caucasian vegetarians.
4. Vitamin A and Vitamin C intakes were adequate in all groups. The Indians had significantly lower intakes of Vitamin D and Vitamin E.

5. The intake of 'B' group vitamins was adequate in all the groups, with the exception of vitamin B₁₂ and folate. Vitamin B₁₂ intakes were lower in the vegetarians and the Indians also had lower folate intakes.
6. Calcium and iron intakes were similar in all the groups. Haem iron formed a quarter of the total dietary iron in the omnivorous diet. The Indian vegetarians had significantly lower intakes of copper and zinc compared with the Caucasians.
7. Generally the diets of Caucasian vegetarians were adequate with regard to most nutrients, with the exception of vitamin B₁₂, but the Indian diets appeared to be low in vitamin B₁₂, folate, copper, zinc and α -linolenic acid.

CHAPTER 5

HAEMATOLOGY

5:1 Introduction

5:1:1 Definition of Anaemia

Anaemia is one of the most common problems encountered in clinical medicine and is functionally defined as a decrease in the competence of blood to carry oxygen to tissues thereby causing tissue hypoxia (McKenzie, 1988). However, anaemia is not a disease but rather the expression of an underlying disorder or disease; it is an important clinical marker of a disorder that may be basic or sometimes more complex (Spaet, 1980). It is characterized by decreased red cell mass.

The World Health Organization (WHO) proposed a definition, based on haemoglobin concentrations of different groups of individuals. A WHO group of experts in 1971 defined normal haemoglobin concentration as follows:

"It is recognised that there is a homeostatic mechanism that sets the haemoglobin level in each individual. Whereas it is not known whether this is the optimum level for health, it is accepted as normal for the individual. The distribution of such normal values in the population should be derived from a representative sample of healthy persons in whom the presence of nutritional deficiencies have been excluded by specific laboratory determinations or by prior administration of haemotonic. This distribution of normal values is likely to be the same throughout the world when allowance is made for such factors as age, sex, pregnancy and altitude."

WHO (1972)

TABLE 5:1

Haemoglobin concentrations below which anaemia is likely to be present at sea level

	Hb (g/dl)
<i>Children</i>	
<i>6 months to 6 years</i>	<i>11</i>
<i>6 years to 14 years</i>	<i>12</i>
<i>Adults</i>	
<i>Men</i>	<i>13</i>
<i>Women</i>	<i>12</i>
<i>Pregnant Women</i>	<i>11</i>

Nutritional anaemia can be defined as a condition in which the haemoglobin concentration is below the level that is normal for a given individual, due to deficiency of one or more of the nutrients required for haemopoiesis, and conversely as a condition in which the haemoglobin concentration can be raised by increasing the amount of nutrient(s) absorbed (Baker & DeMaeyer, 1979). The main haemopoietic nutrients are iron, vitamin B₁₂ and folate. There is also evidence that some other nutrients such as protein, pyridoxine, ascorbic acid, copper and vitamin E are necessary for haemopoiesis (Passmore & Eastwood, 1986). Deficiency states of iron, vitamin B₁₂ and folate are mainly implicated in the development of nutritional anaemia.

Anaemias give rise to the same general clinical features, whatever the cause. Symptoms only arise when the transport of oxygen by the blood is insufficient to meet the needs of the body. As the need for oxygen is related to physical activity, a person leading a sedentary life may have a moderate degree of anaemia and yet be entirely free of symptoms, though these develop if unaccustomed exercise is taken. As anaemia often develops slowly, the patient may gradually and unknowingly reduce physical activity, therefore it is not unusual to find a woman undertaking her normal housework with a haemoglobin level of less than 7.5 g/dl, but doing it slowly. The severity of the clinical features depends not only on the degree of anaemia, but on the rapidity of its development. Common symptoms are general fatigue, lassitude, breathlessness on exertion and paraesthesiae.

5:1:2 Iron Deficiency Anaemia

Iron is an essential component of haemoglobin and of the respiratory enzymes. Deficiency of iron results in inadequate haemoglobin production and consequent impairment of red cell formation. About two-thirds of the iron in the body is found in red cells and the rest in myoglobin, respiratory enzymes and as stored iron in the form of ferritin and haemosiderin. In practice, iron deficiency is defined as a state with no iron stores (serum levels of ferritin < 12 µg/l) and with an insufficient supply of iron to haemopoietic tissues. Iron deficiency anaemia is the state when this process led to a haemoglobin level below the lower limit of normality for the relevant population group (11 g/dl in the population of this study).

The prevalence of iron deficiency has been studied in many countries. It has been estimated that at least 800 million individuals in the world have iron-deficiency anaemia (WHO, 1990), the prevalence being the highest in Africa and South Asia - 40% in all groups excluding adult males. In Europe the corresponding figure is much lower, about 5-10%. Iron deficiency irrespective of anaemia is prevalent even in developed countries and is relatively common in women of child-bearing age (Jacobs *et al.*, 1969; Rybo *et al.*, 1985). Jacobs *et al.* (1969) investigated a random sample of women in Wales and found that 12% of the women had iron depletion alone and 10% of the women also had iron deficiency anaemia.

The high prevalence of iron deficiency anaemia correlates better with the type of diet than with total iron intakes. Absorption of iron is not only dependent on the total iron present in the diet, but also on the form of iron ingested and the food mixture eaten. Dietary iron exists in two forms: haem iron present primarily in red meats, and non-haem iron present in vegetables and whole grains. Non-haem iron is not easily absorbed due to the presence of dietary fibre, carbonates, oxalates, phosphates, phytates and tannates which form insoluble complexes with iron and limit its absorption (International Nutritional Anaemia Consultant Group, 1982). Although non-haem iron represents the majority of dietary iron, only 2% of this form is actually absorbed in the duodenum (Conrad and Barton, 1981). Therefore, iron deficiency is most prevalent in populations in developing countries subsisting on predominantly vegetarian cereal and legume diets (Hallberg, 1981). Iron deficiency is the major nutritional problem in India (National Institute of Nutrition, 1980) and also has been reported in Indian immigrant populations residing in South Africa, U.S.A., Canada and the U.K. (Ganapathy and Dhanda, 1980; MacPhail *et al.*, 1981; Robertson *et al.*, 1982; Bindra and Gibson, 1986). It has been generally concluded that Caucasian vegetarian diets are nutritionally adequate and studies in long-term vegetarians (Seventh Day Adventists) have reported satisfactory iron status (Anderson *et al.*, 1981). On the other hand, Helman and Darton-Hill (1987) found new vegetarian (Caucasians) women at risk of developing iron deficiency.

The onset of iron deficiency anaemia is insidious, usually occurring over a period of months to years, but precipitating factors such as sudden blood loss, pregnancy or any other physiological stress may shorten the time for deficiency to develop into classic microcytic hypochromic anaemia. Clinical symptoms may be absent during the early

stages of iron deficiency when the body stores are mobilised, but on depletion of iron stores, symptoms of hypoxia (lethargy and weakness) appear. When anaemia is mild the morphology of red cells is little affected and with progressive severity the characteristic features of maturation defect appear. Microcytosis (small cell size) and anisocytosis (increased variability in cell size) resulting in increased red cell distribution width (RDW), are usually the first morphologic changes. It is believed that microcytosis precedes hypochromia (McKenzie, 1988). Diagnosing early stages of iron deficiency is made difficult by the absence of clear clinical symptoms and is often undetected until precipitating factors such as pregnancy, stress or blood loss are encountered.

5:1:3 Macrocytic Anaemia

Macrocytic anaemias are characterised by large red cells with a normal haemoglobin content. These are generally classified as megaloblastic or non-megaloblastic, depending on the morphologic characteristics of erythrocyte precursors in the bone marrow. Megaloblastic anaemias are the result of abnormal DNA synthesis (a nuclear maturation defect). The basis for non-megaloblastic anaemias is not well defined but it has been suggested that macrocytes may be caused by an increase in membrane lipids or to a delay in maturation. Alcoholism, liver disease and reticulocytosis are most commonly associated with non-megaloblastic macrocytic anaemia, where the red cells are thin and round with an increased diameter but with a normal MCV. In contrast, megaloblastic macrocytes are thick and oval with both an increased diameter and an increased MCV.

Deficiency of folate and vitamin B₁₂ which are involved in the maturation of erythrocytes leads to megaloblastic anaemia. This may be either due to dietary lack or much more commonly in Europe and North America, a defect in intestinal absorption. Megaloblastic anaemia is a common problem in areas where Hindu vegetarians are numerous. Most cases are considered to have nutritional deficiency of vitamin B₁₂, but folate deficiency has also been implicated (Mathews and Wood, 1984). Combined dietary deficiency of both folate and vitamin B₁₂ are usually associated with megaloblastic anaemia. Folic acid (pteroylglutamic acid) is the parent substance of a large group of compounds known as folates. It is converted into an active form - tetrahydrofolate (THF) in the body by reduction and this reaction is mediated by vitamin B₁₂. THF acts as a coenzyme in the transfer of single carbon atom groups (such as formyl, CHO;

fomino $\text{CH} = \text{NH}$; and methyl CH_3) in amino acid metabolism and in purine and pyrimidine synthesis for the formation of RNA and DNA.

Vitamin B_{12} acts as a coenzyme methyl cobalamin which catalyses a transmethylation from methyl-THF to homocysteine to form methionine and thus releases THF for other single carbon transfer reactions important to nucleic acid synthesis. This reaction is a site of vitamin B_{12} -folate interaction and may relate to the similarity in vitamin B_{12} and folate deficiency signs (Herbert and Colman, 1988). Therefore, lack of vitamin B_{12} may limit DNA synthesis by trapping folate as methyl-THF and consequently result in slower replication of cells.

The other cobalamin coenzyme, deoxyadenosyl cobalamin, catalyses the conversion of methylmalonyl-coenzyme A to succinyl coenzyme A, a reaction in the pathway for the degradation of certain amino acids and odd-chain fatty acids. Blockage of this reaction in B_{12} deficiency leads to the characteristic increased urinary excretion of methyl-malonic acid and may also result in demyelination of nerve fibres. Demyelination is a consequence of erroneous fatty acid synthesis due to accumulation of propionyl CoA which is used as a primer for fatty acid synthesis instead of acetyl CoA in the absence of vitamin B_{12} . This results in the synthesis of fatty acids with odd number of carbons which are incorporated into neuronal membrane, thus causing disruption of membrane function. A critical feature of demyelination in vitamin B_{12} deficiency is neurological disease. Peripheral nerves are most often affected presenting as motor and sensory neuropathy. The brain and spinal cord may also be affected leading to dementia, spastic paralysis and other neurological disturbances such as sub-acute combined degeneration of the spinal cord. Neurological symptoms are not seen in folic acid deficiency. Deficiency of vitamin B_{12} in the presence of adequate folate status may prevent macrocytosis but still allow the insidious progress of neurological symptoms in an individual.

5:1:4 Diagnosis and Classification of Anaemia

The most common complaint in anaemia is tiredness, muscle weakness and fatigue due to insufficient oxygen available for energy production. Although specific diagnosis is the ultimate goal of any anaemia classification system, it is important to remember that anaemia frequently develops from more than one mechanism, complicating correlation

and interpretation of results. Also, additional complicating factors may alter the typical findings of a specific anaemia.

Anaemias may be initially classified according to the average size and haemoglobin concentration of the red blood cells and indicated by the following red cell indices.

1. *Red blood cell (RBC) or erythrocyte count:* Erythrocytes comprise about 35-45% of blood volume and the concentration varies with sex, age and geographic location. Males have higher erythrocyte concentrations than females, the range being $4.2-5.9 \times 10^{12}/l$. A decreased red cell count is indicative of anaemia.
2. *Haemoglobin:* Haemoglobin concentration is an indirect measure of oxygen-carrying capacity of the blood. Concentrations below 12 g/dl in non-pregnant women are considered to indicate anaemia, but in this study a level of 11 g/dl has been used as a cut-off point.
3. *Packed cell volume (PCV) or haematocrit:* The volume of packed red blood cells after centrifugation in relation to the volume of whole blood expressed in litre/litre is known as haematocrit or PCV. The reference ranges for PCV are 0.30-0.47 l/l
4. *Red cell indices:* These are useful aids in classifying the erythrocytes as to their size and haemoglobin content. Since abnormal morphology is typical of some types of anaemia, the indices are used for classification of anaemic states.
 - (a) Mean cell volume (MCV) indicates the average volume of individual red cells in femtolitres (fl) and is the ratio of PCV to erythrocyte count. The MCV is used to classify cells as:

Normocytic:	MCV 74-99 fl
Microcytic:	MCV < 74 fl
Macrocytic:	MCV > 99 fl
 - (b) Mean corpuscular haemoglobin concentration (MCHC) is the average concentration of haemoglobin in grams in a decilitre of red cells and is the

ratio of haemoglobin to PCV. This index compares the average concentration of haemoglobin within individual cells to the average volume of the cell and indicates whether the general erythrocyte population is:

Normochromic: 29-36 g/dl, or

Hypochromic: <29 g/dl

- (c) Mean corpuscular haemoglobin (MCH) is a measurement of average weight of haemoglobin in individual red cells and is calculated as follows:

$$\text{MCH (pg)} = \frac{\text{Haemoglobin (g/dl)}}{\text{RBC count (x10}^{12}/\text{l)}} \times 100$$

MCH does not take into account the size of a cell and therefore it is not a particularly useful index.

- (d) Red cell distribution width (RDW) is the coefficient of variation of red cell volume distribution (standard deviation/mean MCV x 100). RDW greater than 16% indicates an increase in the red cell size or anisocytosis. RDW must be interpreted cautiously because an increased standard deviation (variability) with an increased MCV may give a normal RDW; conversely a normal deviation (little variability) with a decreased MCV may give an increased RDW.

Anaemia may be initially classified according to the morphology of the red cells as described above, but it may be insufficient in determining the functional basis of anaemia. For example, a combined deficiency of iron and folate may result in normal MCV even though iron deficiency is normally microcytic and folate or B₁₂ deficiency is macrocytic. Anaemia is characterized by disturbances in cell proliferation and maturation and further investigation into the individual's status of haemopoietic nutrients such as iron, vitamin B₁₂ and folate would give vital clues as to the aetiology and pathophysiological mechanisms involved in its development. During iron deficiency cells divide normally but are low in haemoglobin and hence hypochromic and microcytic with lower red cell indices of MCH, MCHC and MCV. However, in vitamin B₁₂ or folate deficiency the cells are unable to divide or proliferate and therefore large cells are seen in the blood film but may have normal haemoglobin concentrations and MCV could be raised but MCH may be normal. Dietary intakes of omnivores, Indian and Caucasian vegetarians varied with regard to vitamin B₁₂ and folate. Although total iron intakes were similar, their sources were different. This chapter compares the dietary intakes,

biochemical indices of iron, vitamin B₁₂ and folate status and haematological status in these groups of pre-menopausal women.

5:2 Methods

Analytical methods used are described in detail in Chapter 2. Data were analysed by one-way analysis of variance. Data that were not normally distributed were log-transformed before analysis. The results of data that were log-transformed are expressed as geometric means with 95% confidence intervals, otherwise the data is presented as means \pm SE. Statistical correlations were made using the product-moment correlation. The Chi square test was used to test for differences between groups in the proportion of individuals with certain characteristics outside the normal range.

5:3 Results

Blood samples were obtained from twenty-two Caucasian omnivorous women, twenty-three Indian vegetarian and eighteen Caucasian vegetarians and dietary intakes from twenty-two, twenty-one and eighteen subjects, respectively. Detailed analysis of dietary intakes are given in Chapter 4. Two omnivores, four Indian vegetarians and four Caucasian vegetarians were taking a multi-vitamin and mineral supplement.

Haemoglobin concentrations were generally inside the normal ranges, with the exception of two Indian vegetarian subjects (10.8 and 10.9 mg/dl). However, several differences in haemoglobin concentration and blood counts were noted between the three groups (Table 5:2). Haemoglobin concentration, packed cell volume, mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were significantly lower and platelet counts were significantly higher in the Indian women compared with caucasian omnivores. Erythrocyte counts (RBC) and erythrocyte distribution width (RDW) were significantly lower and MCV and MCH significantly greater in Caucasian vegetarians compared with the omnivores and MCHC was significantly greater compared with that of the Indians. The intake of supplements did not alter the statistical significance observed for blood counts and haemoglobin concentrations. However, the mean concentrations of haemoglobin were lower when subjects taking supplements were excluded from the analysis, but within the normal ranges.

TABLE 5:2

Blood counts and haemoglobin concentrations

	Caucasian Omnivores (n=22)		Indian Vegetarians (n=23)		Caucasian Vegetarians (n=18)	
	Mean	SEM	Mean	SEM	Mean	SEM
WBC ($\times 10^9/l$)	6.0	0.37	6.2	0.28	5.7	0.28
RBC ($\times 10^{12}/l$)	4.7 ^a	0.10	4.6 ^a	0.07	4.4 ^b	0.07
Hb (g/dl)	13.6 ^a	0.20	12.6 ^b	0.23	13.6 ^a	0.17
PCV (l/l)	0.41 ^a	0.006	0.39 ^b	0.007	0.40 ^a	0.005
MCV (fl)	88.8 ^a	1.52	84.7 ^b	1.16	92.7 ^a	0.89
MCH (pg)	29.5 ^a	0.70	27.7 ^b	0.47	31.2 ^a	0.36
MCHC (g/l)	331.0 ^{ab}	3.3	327.0 ^b	1.7	337.0 ^a	1.8
RDW (%)	15.1 ^a	0.61	16.3 ^a	0.55	12.3 ^b	0.15
Platelets ($\times 10^9/l$)	256.0 ^b	11.2	311.0 ^a	14.5	279.0 ^{ab}	18.2
MPV (fl)	8.2	0.36	7.9	0.17	8.3	0.19
Neutrophils ($\times 10^9/l$)	3.5	0.25	3.6	0.19	3.7	0.27
Lymphocytes ($\times 10^9/l$)	1.9 ^{ab}	0.12	2.0 ^b	0.14	1.6 ^a	0.07
Monocytes ($\times 10^9/l$)	0.36	0.032	0.35	0.022	0.34	0.023

^{ab,c}: Values with unlike superscript letters were significantly different ($p < 0.05$).

All the subjects seemed healthy and none complained of any symptoms of anaemia, except one Indian subject who was prescribed iron supplements by her doctor. One omnivorous subject had low MCV (68.5 fl), MCH (21 pg) and haemoglobin (11.1 mg/dl) and raised RDW (23 %) and was diagnosed as anaemic with microcytosis, although levels of serum vitamin B₁₂, folate and ferritin were within normal ranges. This subject was of Italian origin and target cells were also observed in the blood film indicating impaired haemoglobin production. The two Indian subjects with haemoglobin concentrations lower than 11 mg/dl showed a raised RDW, but only one subject showed reduced MCV and MCH. Eleven Indian women had RDW greater than the upper limit of normality (16 %), as compared with eight omnivores. None of the caucasian vegetarians had RDW outside the normal range (Table 5:3).

TABLE 5:3

Number of subjects outside the normal ranges for haematological values (total number of subjects given in parentheses)

	Caucasian Omnivores	Indian Vegetarians	Caucasian Vegetarians	Statistical Significance
Hb (<110 g/l)	0 (22)	2 (23)	0 (18)	NS
MCV: <74 fl >99 fl	1 (22) 1 (22)	1 (23) 0 (23)	0 (18) 1 (18)	NS NS
RDW (>16%)	8 (22)	11 (23)	0 (18)	$p<0.01$
Serum Ferritin (<12 µg/l)	6 (22)	15 (19)	10 (18)	$p<0.01$
Serum Vitamin B ₁₂ (<180 ng/l)	0 (22)	9 (21)	9 (18)	$p<0.01$
Serum Folate (<2 µg/l) (<3 µg/l)	0 (22) 5 (22)	0 (21) 0 (21)	0 (18) 0 (18)	NS $p<0.01$
Erythrocyte Folate (<200 µg/l)	5 (19)	8 (18)	7 (18)	NS

Hb: haemoglobin; MCV: mean corpuscular volume; RDW: red cell distribution width; NS: not significant.

Statistical Method: Chi-squared test.

The mean serum vitamin B₁₂ concentration was lower in both Indian and caucasian vegetarians than in the omnivores and was associated with low dietary intakes (Table 5:4). This difference became more marked if the subjects taking supplements of vitamin B₁₂ were excluded from the analysis and resulted in similar serum vitamin B₁₂ concentrations in both groups of vegetarians (Figures 5:1a & 5:1b). A high proportion of vegetarian subjects had serum vitamin B₁₂ levels below the normal range (180 ng/l). Four vegetarians (two Indians and two Caucasians) had serum vitamin B₁₂ concentrations below 120 ng/l (Table 5:3), the level below which clinical signs of deficiency usually appear (Matthews and Wood, 1984).

Serum folate concentrations were greater in the Caucasian vegetarians compared with the other groups and reflected intakes of folate. Serum folate concentration is more liable to change with recent dietary intakes, unlike erythrocyte folate which is known to be a reliable indicator of folate status. If subjects taking supplements were excluded from the analysis, the mean erythrocyte folate concentration was significantly lower in the Indian women compared with the other two groups. Five omnivores, eight Indians and seven caucasian vegetarians had erythrocyte folate concentrations below 200 ug/l (Table 5:4 and Figures 5:2a & 5:2b).

Serum ferritin concentrations were significantly lower in both vegetarian groups compared with the omnivores (Figure 5:3a). In the subjects not taking supplements, mean ferritin concentrations in Caucasian and Indian vegetarians were less than half the mean value of 24.7 ug/l (SE 3.92). It is notable that haem iron supplied approximately 25% of total iron intakes of the omnivores (mean 2.83, SEM 0.34) (Figure 5:3b). The intake of haem-iron was positively correlated with serum ferritin concentration ($r=0.59$, $P<0.01$, $n=18$) in omnivorous subjects not taking supplements. Ferritin concentrations in Caucasian and Indian vegetarians were on the borderline of the lower level of normality (12 ng/ml). However, when subjects taking supplements were excluded, mean ferritin concentrations in both vegetarian groups fell below the normal range.

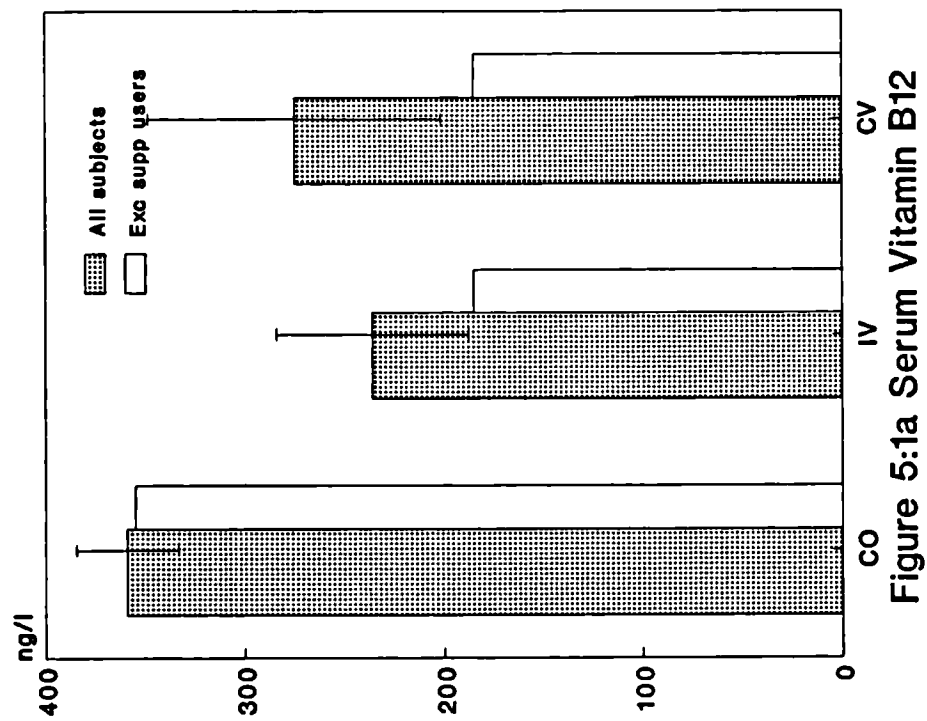


Figure 5:1a Serum Vitamin B12

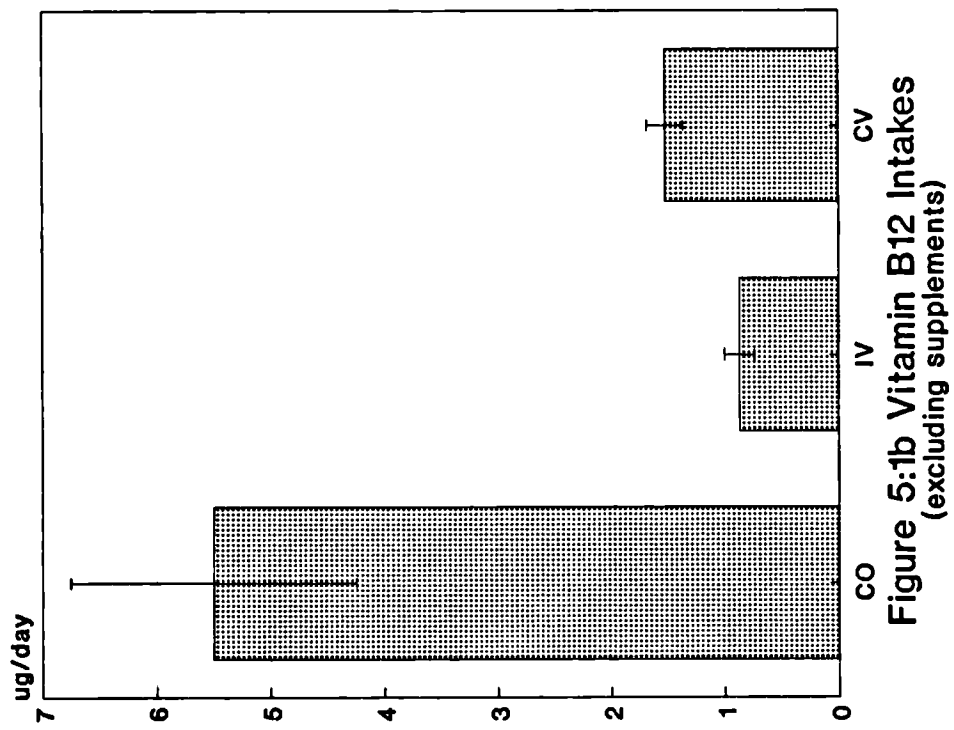


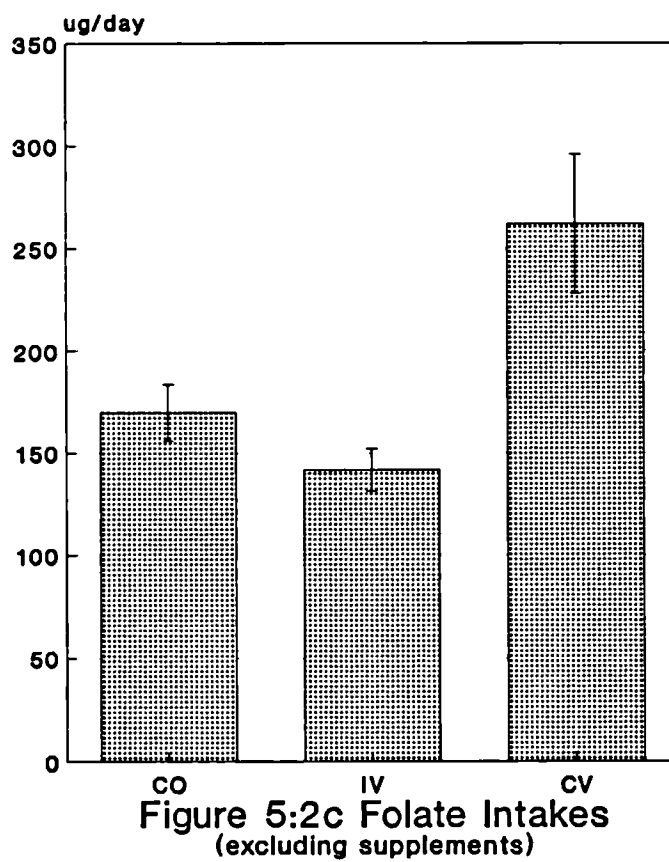
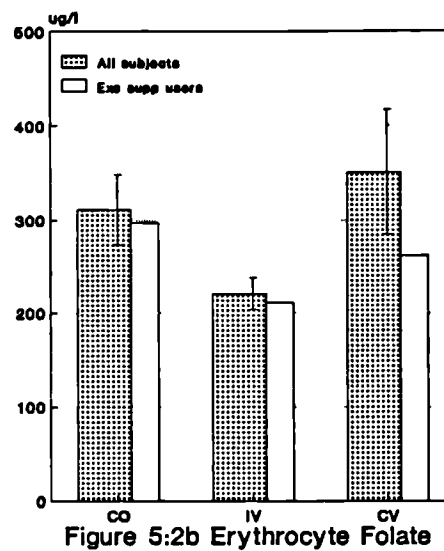
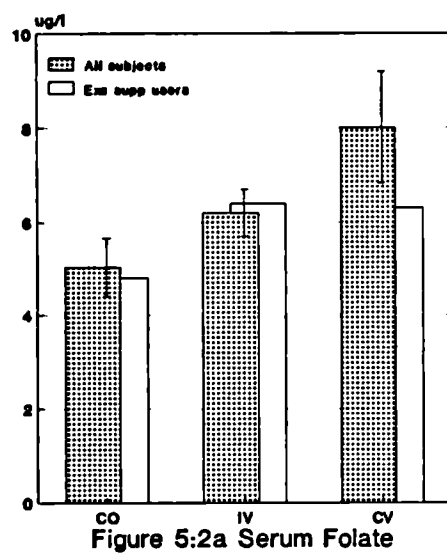
Figure 5:1b Vitamin B12 Intakes
(excluding supplements)

TABLE 5:4

Serum ferritin, vitamin B₁₂, folate and erythrocyte folate concentrations of the subjects (geometric mean (GM) and 95% confidence intervals (CI))

	Ferritin (μ /l)		Vitamin B ₁₂ (ng/l)		Folate (μ g/l)		Erythrocyte Folate (μ g/l)	
	GM	CI	GM	CI	GM	CI	GM	CI
<u>Caucasian Omnivores</u>								
All Subjects (n=22)	20	15.9-25.1	341	265-439	4.43	3.71-5.29	278	253-305
Excluding Supplement Users (n=20)	18	11.2-29.2	336	276-409	4.27	3.55-5.15	273	224-332
<u>Indian Vegetarians</u>								
All Subjects (n=19)	7.9	4.8-12.9	199	159-256	5.85	4.9-6.99	211	174-255
Excluding Supplement Users (n=17)	6.4	3.8-11.8	178	145-219	6.06	4.98-7.38	201	163-248
<u>Caucasian Vegetarians</u>								
All Subjects (n=18)	11.1	6.7-18.4	197	153-253	6.93	5.73-8.4	284	235-344
Excluding Supplement Users (n=14)	10.4	5.8-18.4	158	125-198	5.79	4.66-7.2	230	185-286

Statistical Method: Analysis of variance of log-transformed data.



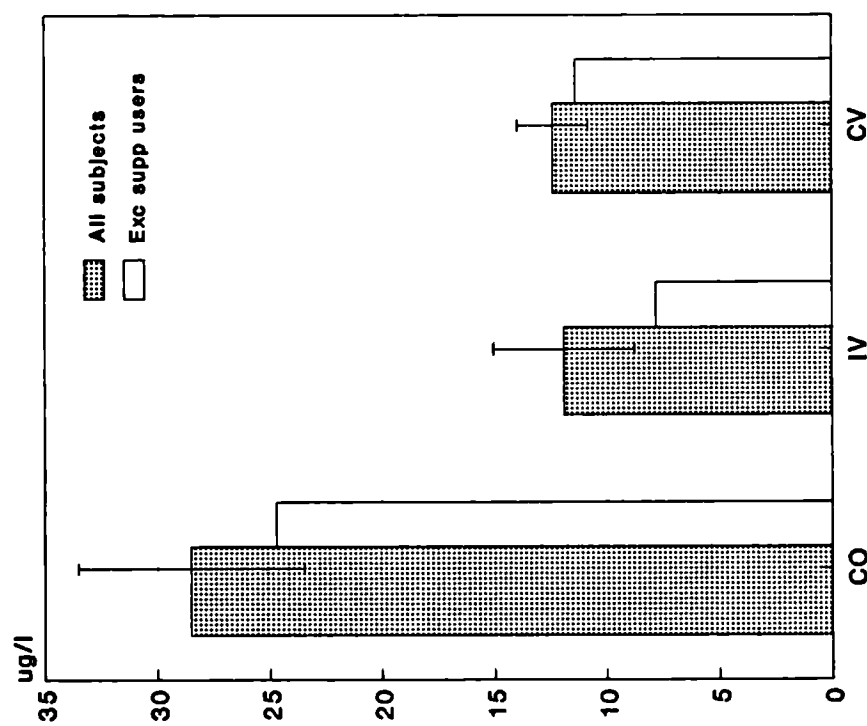


Figure 5:3a Serum Ferritin

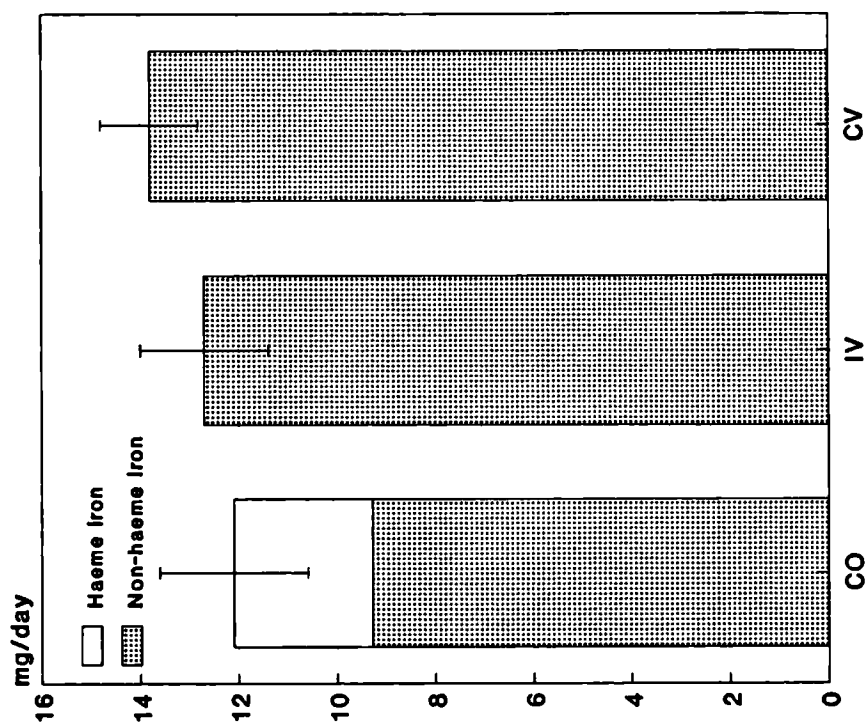


Figure 5:3b Iron Intakes
(excluding supplements)

5:4 Discussion

The intakes of vitamin B₁₂ were low in both vegetarian groups and less than the US recommended daily amounts of 2 µg/d (USA) but only the Indian women had intakes below the UK RNI. The intake of vitamin B₁₂ in omnivores is similar to that reported in British women and was derived mainly from meat (Gregory *et al.*, 1990). It is perhaps surprising that the intakes of vitamin B₁₂ were so low in the vegetarians, in view of the availability of a large number of processed foods supplemented with the vitamin. This might suggest that these supplemented foods are not popular with vegetarians. Although vitamin B₁₂ may have been underestimated because of unknown values in the food tables, the low serum vitamin B₁₂ concentrations in the vegetarian subjects suggests that their dietary intake was inadequate. Several (eighteen) vegetarians had serum vitamin B₁₂ concentrations below the normal range. Vitamin B₁₂ deficiency of dietary origin may take several years to develop owing to liver stores of the vitamin. The risk of vitamin B₁₂ deficiency in vegans, who exclude all food of animal origin, has been recognized for some years (Sanders *et al.*, 1978). The results of this study suggest that vitamin B₁₂ deficiency is a hazard in both Indian and Caucasian vegetarians.

Folate intakes were relatively high in Caucasian vegetarians, but low in the Indian vegetarians. It is well known that high folate intakes inhibit the development of megaloblastic anaemia caused by inadequate intakes of vitamin B₁₂, while permitting the development of the more insidious neurological symptoms of vitamin B₁₂ deficiency such as sub-acute combined degeneration of the spinal cord. It is likely, therefore, that Caucasian vegetarians would present neurological rather than haematological symptoms of vitamin B₁₂ deficiency. However, MCV and MCH were both significantly greater in this group. Vitamin B₁₂ is involved in the transport of folate to the erythrocyte. In vitamin B₁₂ deficiency serum folate concentration can be raised and those of erythrocyte folate depressed. It is notable that although serum folate concentration tended to be slightly greater in the Indian women compared to the omnivores, erythrocyte folate concentrations were significantly lower.

Iron intakes were similar in all three groups and below the UK RNI of 14.8 mg/d. Also, the availability of iron from their diet is variable, while haem-iron derived from meat is well absorbed, iron from plant sources is generally poorly absorbed and substances present in plant foods, such as phytate and fibre, may render the iron

unavailable. It has also been suggested that a high intake of vitamin C may aid iron absorption by conversion of ferric-iron to ferrous-iron. Ferritin concentrations are generally considered to be good indicators of Fe stores (Cook and Skikne, 1982). Ferritin concentrations in both groups of vegetarians were approximately half the value found in the omnivores. It seems likely that this difference can be attributed to the haem-iron present in the diets of the omnivores which provided approximately 3 mg/d. A significant correlation ($r=0.59$, $p<0.01$) was observed between haem-Fe intake and serum ferritin concentrations in the omnivores. Ferritin concentrations were below the normal range in the majority of vegetarian subjects not taking supplements.

Haemoglobin concentrations were significantly lower in the Indian vegetarians than in either the Caucasian vegetarians or omnivores but within the normal range. MCV and MCH values were also lower. If iron deficiency leading to microcytosis co-exists with megaloblastosis, macrocytosis may be masked and the MCV may not be increased (McKenzie, 1988). This may explain the absence of macrocytosis in the Indians, although their biochemical measures of folate and vitamin B₁₂ indicate deficiency. RDW is a measure of erythrocyte heterogeneity and a high value is indicative of a maturation defect of erythrocytes, regardless of MCV or degree of anaemia. Changes in RDW can be detected before abnormal cells can be identified in the blood smear. Although RDW may not aid in diagnosing the type of nutritional anaemia, it is a useful predictor of an individual's susceptibility to anaemia. RDW was higher in the Indian vegetarians than in the other groups, which implies that they are more susceptible to anaemias caused by a maturation defect. As folate, Fe and vitamin B₁₂ status were similar in both vegetarian groups, it may be that some other nutrient is responsible for this. Copper is a possible haemopoietic nutrient and its intake was lower in the Indians. However, it is recognized that copper intakes may have been underestimated. Further studies should consider measuring indicators of copper status. Erythrocyte (RBC) and lymphocyte counts were lower in the caucasian vegetarians and MCV greater. This observation is similar to that made by Sanders *et al.* (1978) in vegans and may indicate hypoproliferation of blood-forming cells.

Although the subjects of the present study were generally in good health and could not be classified clinically as anaemic, it does not follow that they do not differ with regard to risk of anaemia. Precipitating factors such as pregnancy, illness and excessive

blood loss are implicated in the causation of anaemia. Based on the results of this study it could be concluded that both Indian and Caucasian vegetarians are at increased risk of iron-deficiency anaemia and that Indian vegetarian women are also at greater risk of developing nutritional megaloblastic anaemia. This is in agreement with studies carried out on Indian populations in other developed countries (Ganapathy and Dhanda *et al.*, 1980; McPhail *et al.*, 1981; Bindra and Gibson, 1986). There is clearly a need for nutritional advice targeted at Indian vegetarians.

Meat, especially red meat, plays an important role in supplying iron and vitamin B₁₂ in the average British diet. Individuals who change to a vegetarian diet need to ensure that they have adequate intakes of these nutrients. Wholemeal cereals and dark green leafy vegetables are good sources of iron, but the iron from these sources is poorly absorbed. Vitamin B₁₂ is generally absent from plant foods unless they are contaminated with micro-organisms that produce the vitamin. Milk products are not particularly good sources of vitamin B₁₂, but eggs are. Non-animal sources of vitamin B₁₂ are added to several processed foods such as marmite and cornflakes. However, these may not be consumed by those vegetarians who avoid processed foods. These individuals may well benefit from taking supplements.

5:5 Summary

1. Full blood counts, serum ferritin, vitamin B₁₂ and folate, and erythrocyte folate concentrations were estimated in Caucasian omnivores, Indian and Caucasian vegetarians.
2. Haemoglobin concentrations were generally inside the normal range in all groups but were lower in the Indians, as were MCV and MCH. Higher MCV, MCH and lower erythrocyte counts were observed in Caucasian vegetarians compared with the omnivores.
3. In both groups of vegetarians, concentrations of vitamin B₁₂ were lower and related to their dietary intakes. Ferritin levels were also lower in the vegetarians, despite similar intakes of iron, compared with the omnivores. The Indian vegetarians also had lower folate status as measured by erythrocyte folate concentrations.
4. It is concluded that both groups of vegetarians need to ensure they have adequate intakes of iron and vitamin B₁₂.

CHAPTER 6

A COMPARISON OF DIETARY FATTY ACID INTAKE WITH FATTY ACID COMPOSITION OF PLASMA LIPIDS

6:1 Introduction

6:1:1 Dietary Fatty Acids

Fatty acids are usually made up of even numbers of carbon atoms in a straight chain, C2-C38. They can be classified according to chain length, degree and geometry of unsaturation, on which their metabolic fate in the body depends. Certain polyunsaturated fatty acids cannot be synthesized in mammalian tissue and need to be provided in the diet. These are linoleic acid (18:2n-6) and linolenic acid (18:3n-3) and their respective derivatives (Figure 6:1), while all other fatty acids are regarded as non-essential.

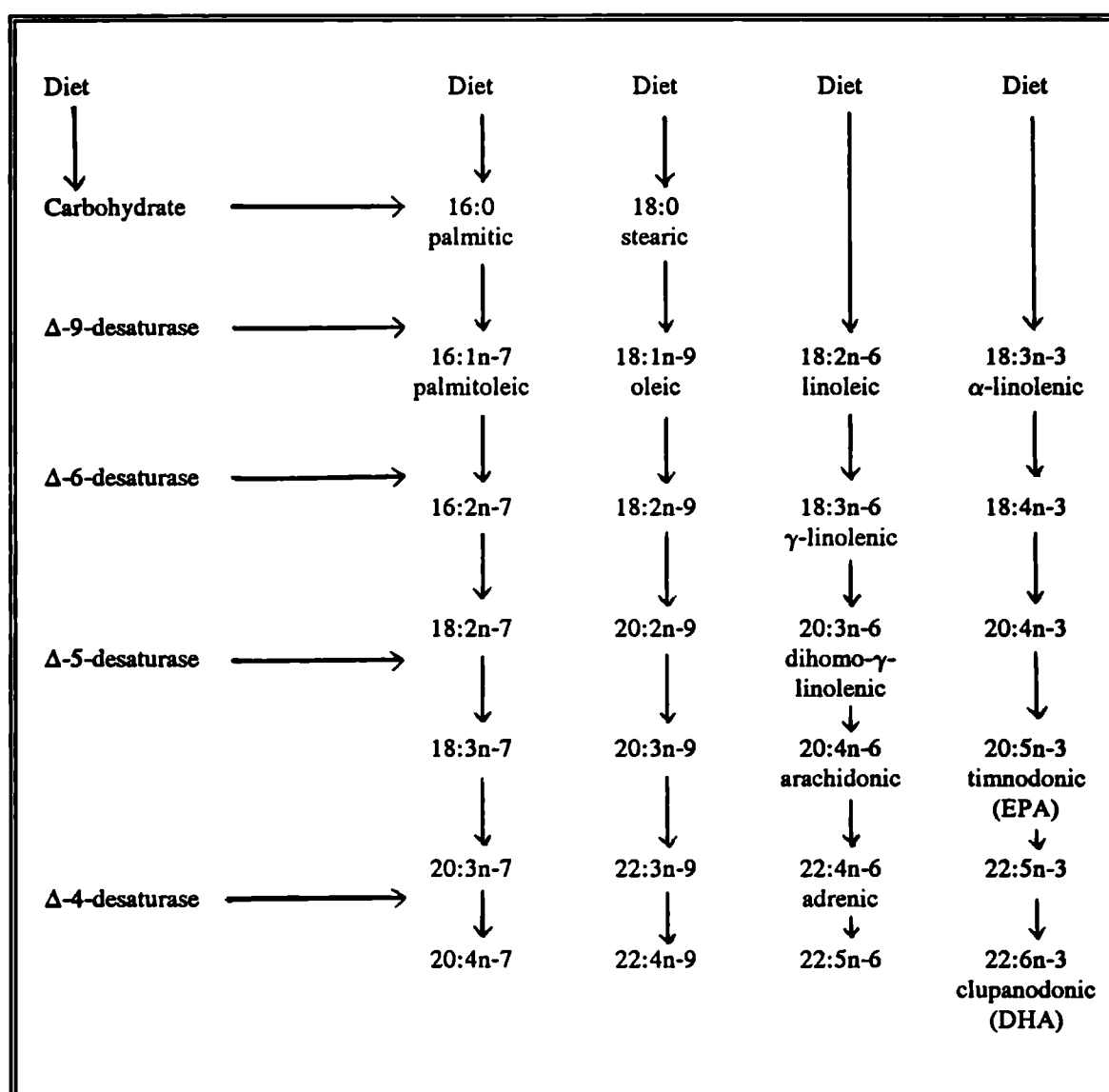


Figure 6:1

Outline of the pathways of metabolism of the n-7, n-9, n-6 and n-3 unsaturated fatty acids and points of action of desaturases

The amount of linoleic acid required to prevent signs and symptoms of deficiency is in the order of 1% of the total energy intake. A dietary requirement for n-3 fatty acids seems likely owing to the concentration of DHA (22:6n-3) in the brain and photoreceptor membranes of the eye. Estimates suggest that 0.5% of the dietary energy as α -linolenic acid may be sufficient. However, DHA may need to be obtained preformed in the diets of pre-term infants.

Several biochemical indicators of essential fatty acid status have been proposed. The presence of 20:3n-9 synthesized through oleic acid (18:1n-9) in the plasma and cell lipids indicates an inadequate intake of polyunsaturates. The ratio of 20:3n-9 to 20:4n-6 (triene : tetrene ratio) in plasma phospholipids has been considered a good biochemical index of essential fatty acid status. A ratio of >0.4 is an indication of deficiency and a ratio of 0.2 has been suggested as the upper limit of normality.

6:1:2 Dietary Sources of Essential Fatty Acids

Linoleic acid is usually the most predominant PUFA in the human diet. Foods from plant sources, such as culinary oils, have a considerable amount of linoleic acid ranging from 40-75% of the total fatty acids; sunflower oil (65%), safflower oil (74%), while olive oil contains only 11%, with other vegetable oils being intermediate. α -Linolenic acid is a minor constituent except in linseed oil, soyabean oil and rapeseed oil, ranging from 7-10% of the total fatty acids. The fatty acid composition of simple-stomached animals reflects the composition of dietary fat, unlike that of ruminants, where owing to the bio-hydrogenation of polyunsaturated fatty acids could result in higher amounts of saturated fatty acids irrespective of the type of dietary fat. Fatty acid composition of animal fat in the human diet varies with the source of meat consumed. However, the phospholipid and cholesterol esters from animal tissues contain derivatives of linoleic (18:2n-6) and linolenic acid (18:3n-3), notably arachidonic acid (20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). Eggs and offal, especially liver and brain, contain significant amounts of C20-C22 polyunsaturated fatty acids but fish and fish oils are by far the best sources of EPA and DHA. Clinical essential fatty acid deficiency is not recognised as occurring naturally in man consuming self-selected diets and is regarded as iatrogenic, occurring under abnormal circumstances such as infants fed on skimmed milk, kwashiorkor, chronic fat malabsorption and in total parenteral nutrition.

6:1:3 Metabolism of Essential Fatty Acids

The parent fatty acids in the diet can undergo further desaturation and elongation via the same pathways to give two series of derivatives (Figure 6:1), the n-6 and n-3 series, but the two series are biologically distinct since there is no interconversion between them (Sprecher, 1977). The rate of elongation proceeds faster than the rate of desaturation for any given fatty acid, such that the delta-5 and delta-6 desaturases are considered the rate-limiting enzymes in PUFA metabolism. These enzymes are regulated by both diet and hormone status and a diurnal rhythm exists (Brenner, 1982).

The major derivative of the n-6 series is arachidonic acid and those of n-3 series are EPA and DHA (Figure 6:1). There is further elongation of 22:6n-3 in the retina to give 32:6n-3 and 36:6n-3 (Avelandano, 1987). In the absence of PUFA in the diet, oleic acid (18:1n-9) can be synthesized from carbohydrate in mammalian tissue and can be converted to eicosatrienoic acid (20:3n-9).

The n-3 and n-6 series share a common enzyme system, located in the microsomal fraction of the cell, and competitive inhibition occurs between them. Linolenic acid is the preferred substrate only if both linoleic and linolenic acids are present in similar amounts in the diet, but this preference can be overcome by increasing the concentration of linoleic acid. Since linoleic acid is more abundant than linolenic acid in the human diet, the major derived PUFA occurring in the tissue lipids is arachidonic acid. The extent to which the parent EFAs are converted to more unsaturated derivatives is dependent upon their affinity for other metabolic pathways. The rate of oxidation of PUFA and their affinity for acyl transferases are influenced by the degree of unsaturation and chain length, so that linoleic acid is oxidised faster than linolenic or arachidonic acids. Oxidation of 18:3n-3, however, occurs at a greater rate than 18:2n-6, possibly because 18:3n-3 is not easily incorporated into phospholipids.

Elongation and desaturation of EFA occurs primarily in the liver and transport of arachidonate to individual cells occurs by esterification into cholesterol and phospholipids of plasma lipoproteins. Arachidonic acid occupies the 2-acyl position in the cell membrane phospholipids and is available for eicosanoid synthesis. When n-3 fatty acids are included in the diet, EPA and DHA compete with arachidonic acid for a position in the membranes, so reducing plasma and cellular levels of arachidonic acid and

consequently altering the related eicosanoid synthesis. Eicosanoids derived from arachidonic acid have a pro-aggregatory effect on platelets whereas those from the n-3 series are weakly pro-aggregatory. This nature of eicosanoids is important in the development of atherosclerosis through the thrombogenic or thrombolytic pathways. Although influenced by the balance of n-6 and n-3 fatty acids in the diet, modification of membrane lipids is regulated and there is specificity within different tissues; for example, DHA is concentrated in brain and retina whereas arachidonic acid is the major PUFA in platelet lipids.

It is apparent that the balance between n-6 and n-3 fatty acids needs to be considered. It has been suggested that 10-25% of the total polyunsaturated fatty acid intake should be derived from n-3 fatty acids. The ratio of linoleic to linolenic acid in the diet influences the proportion of C20-C22 n-6 and n-3 fatty acids in plasma phospholipids. It is also influenced by the intake of pre-formed C20-C22 n-6 and n-3 fatty acids and the level of DHA in plasma phospholipids is primarily determined by the intake of pre-formed DHA (Sanders and Younger, 1981). In experimental animals an increase in the ratio of 18:2n-6 : 18:3n-3 in the diet has been found to result in an increase in 22:5n-6 and decrease in 22:6n-3 in brain phospholipids, but there is no such evidence in humans. However, a ratio of 5-10 : 1 has been recommended by FAO/WHO and in Canada and Sweden. The mean ratio in diets of UK adults is 7:1.

Table 6:1 summarises the fatty acid intakes reported by various studies. Saturated and *trans* fatty acid intakes made up approximately half of the total fatty acids, while polyunsaturated fatty acid intake ranged from 10-16% of the total dietary fatty acids. Intakes of saturated fat have been found to be significantly higher in men (15.4% of total energy) than in women (16.5% of total energy) (Gregory *et al.*, 1990).

The results of the National Food Survey show the trends in fatty acid intakes in the UK. Although the consumption of total fat has fallen over the past 30 years, the proportion of total energy from fat has changed little (42%) and remained above the then COMA recommendation of 35% of energy. However, the consumption of saturated fat has declined to 17.1% in 1989 but is still above the target of 15% recommended by COMA. The P:S ratio has also increased from 0.17 in 1959 to 0.37 in 1989.

TABLE 6:1 Comparison of the results of dietary surveys - average daily intakes of fatty acids (g) and fatty acids as a percentage of total fatty acids (%)

	Adult Survey 1986-1987 2,197 adults aged 16-64			NFS 1988	Caerphilly & Speedwell 1979			Edinburgh /Fife 1980 & 1982	Edinburgh 1976	Northern Ireland 1986-1987
	Men	Women	Both Sexes		C 665 Men aged 45-59	C 49 Women aged 40- 59	S 93 Men aged 45-59			
Total Fatty Acids (g)	94.8	68.2	81.3	86.3	97.0	71.0	98.0	104.2	108.1	97.0
Fatty Acids g(%) Saturated	42.0 (44)	31.1 (46)	36.5 (45)	38.3 (44)	46.0 (47)	33.0 (47)	46.0 (47)	49.0 (47)	53.0 (49)	44.0 (45)
Trans fatty acids	5.6 (6)	4.0 (6)	4.8 (6)	-	-	-	-	-	-	-
Monounsaturated* fatty acids	31.4 (33)	22.1 (32)	26.7 (33)	33.8 (39)	38.0 (39)	28.0 (39)	38.0 (39)	41.5 (40)	42.9 (40)	37.0 (38)
Polyunsaturated* fatty acids, of which	15.8 (17)	11.0 (16)	13.3 (16)	14.2 (17)	13.0 (13)	10.0 (14)	14 (14.0)	13.7 (13)	12.2 (11)	16.0 (17)
Linoleic	13.8 (15)	9.6 (14)	11.7 (14)	-	9.6 (10)	7.3 (10)	10.2 (10)	10.4 (10)	9.3 (9)	-
Linolenic	2.0 (2.1)	1.4 (2.1)	1.6 (2.0)	-	1.3 (1.3)	1.1 (1.5)	1.3 (1.3)	1.4 (1.3)	1.5 (1.4)	-

* Adult survey MUFA values include cis-MUFA only. The trans-MUFA are included in the value for trans fatty acids

b Adult survey PUFA are classified as n-3 or n-6 in the report. It has been assumed for this table that all n-6 PUFA is 18:2 and all n-3 PUFA is 18:3

6:1:4 Assessment of Fatty Acid Intakes

In order to study the influence of dietary fat on health it is necessary to assess fat intakes both quantitatively and qualitatively. In practical dietetics it is necessary to refer to 'visible fats' such as butter, margarine, vegetable oils, etc. which are added to foods during processing or cooking, mainly to enhance palatability. These can be easily and accurately measured in individual diets. In contrast, many other foods contain proportions of fat, closely associated with other constituents, in an emulsion or as part of a tissue.

Different samples of the same food may vary widely in fat content, especially in the case of meat, although plant foods are relatively constant in their lipid content. Moreover, fat from animal foods varies in quality depending on the nature of fat in the animal's diet prior to slaughter, especially fat from poultry and pork. Such variability within the same foods makes it difficult to estimate fat intakes accurately despite the approximate figures provided by food tables and nutritional labelling. Therefore chemical analysis of the diet actually consumed may be the only reliable means of assessing both the quantity and quality of fat in the diet (Roshanai and Sanders, 1984). However, chemical analysis is expensive, time-consuming and is impractical in large dietary surveys.

Biological Markers of Fat Intake

There is no satisfactory marker of total fat consumption. Low density lipoprotein-cholesterol (LDL-cholesterol) has been used in cardio-vascular studies as a marker of high/low intake, but its relation to total fat intake is weak and is related to the type of fat usually consumed in a given population. Apo AIV has been suggested as a marker for total fat intake but it seems to be only of value in the short term.

Some good markers of qualitative characteristics of dietary fats are available. Depot fat and structural lipids are the two forms in which lipids are found in the cells of humans, the former in adipose cells which contain a large amount of neutral fat, mainly triglycerides, in their cytoplasm. Structural lipids are an essential component of cell membranes and, depending on the type of tissue, they are present in the form of phospholipids, sphingomyelins and glycolipids. The basic constituents of both triglycerides and structural lipids are fatty acids and humans have several metabolic

pathways for transforming and synthesizing fatty acids, with the exception of linoleic and linolenic acids which, being essential, must be provided by diet. Therefore the content of EFA and their derivatives in human tissues and fluids depends more on dietary intake than other fatty acids that can be synthesized in the body. Hence, the fatty acid compositions of these tissues and fluids could be assumed to be reasonable biological markers of the quality of dietary fat.

Triglyceride Fatty Acids in Depot Fat: Adipose Tissue

It is well established that the fatty acid composition of diet strongly influences the type of triglyceride fatty acid stored in adipose tissue. Fat absorbed by the gut reaches the adipose tissue either directly in the form of chylomicrons, or from the liver as very low density lipoproteins. Triglycerides can also be synthesized in the adipose tissue from glucose. Adipose tissue represents 15-30% of body weight in normal adults, the amount being higher in women (10-20 kg) than in men (8-15 kg) as a consequence of complex metabolic and hormonal mechanisms which regulate the quantity as well as the anatomical distribution of fat deposition. The turnover of fatty acids in adipose tissue is slow (Hirsh *et al.*, 1960), especially in individuals where body weight and nitrogen balance remain stable, the half-life of fatty acids in adipose tissue storage is in the order of 600 days (Beynen *et al.*, 1980). This implies that dietary changes in the proportions of different types of fatty acids (such as increase in polyunsaturated to saturated fatty acids) would take one to two years to become apparent from the analyses of adipose tissue fatty acids, and about three years before being completely reflected by the biological marker (Turpeinen, 1973).

When the intake of fat is large and the subject is gaining weight, the deposition of fat in adipose tissue is increased and any change in dietary fatty acids will be reflected quickly in the composition of adipose tissue. On the other hand, when an individual is losing weight, changes in dietary fatty acids take longer to appear in adipose tissue because fat deposition is slowed down and there is increased catabolism of fat to meet energy needs. A poor correlation is found when fat intake is compared over a short period of a few days with fatty acid composition from adipose tissue biopsy (Plakké *et al.*, 1983). However, if the period of dietary assessment is long enough (19 days or more), the correlation of dietary fatty acids and fat biopsy is favourable (von Staveren *et al.*, 1986). Analysis of subcutaneous adipose tissue is a useful marker of the quality

of dietary fat in the long-term but is limited by its doubtful feasibility and acceptability by the subjects (Beynen and Katan, 1985). However, some studies have shown that the subjects found fat biopsy to be only slightly more painful than venipuncture (Smith *et al.*, 1986; Callmer, 1987).

Phospholipid Fatty Acids in Cell Membranes

Phospholipids are essential constituents of cell membranes, representing 97% of erythrocyte membrane lipids, and vary according to the type of cell, its function and the type of membrane. Fatty acids enter into the composition of cell membranes as a component of complex phospholipid molecules. The determination of fatty acid composition of erythrocyte membranes is used for investigating dietary fat intake (Horwitt, 1959) and to monitor changes in dietary pattern (Farquhar and Ahren, 1963), essentially in studies to investigate the relationship between diet and cardiovascular disease. This method is also useful to measure compliance with dietary recommendations implying changes in the type of dietary fat (Angelico *et al.*, 1982). The method has many practical advantages over adipose tissue biopsy in that subjects accept venipuncture more easily and it does not require specially trained staff or equipment. Fatty acid composition of platelets have also been used as an index of fat intake in addition to adipose tissue biopsy (Wood, 1984), especially to investigate the effect of fatty acid composition as a modifier of platelet function in relation to the formation of thrombosis (Renaud *et al.*, 1986). McMurchie *et al.* (1984) have proposed a non-invasive method to investigate fatty acid composition of tissues from cheek cell membranes because of their rapid turnover of approximately five days and the type of fatty acids incorporated in cheek cell membranes should be an indicator of dietary intake over a short period of time. Cheek cells can be easily obtained by rinsing the mouth with distilled water and collecting the fluid. However, this method was found to reflect an increase in P:S ratio over the six week period, but failed to show any appreciable changes in fatty acid composition when the P:S ratio was decreased over a similar period.

Fatty Acids in Plasma

Fatty acids are present in plasma mainly as components of triglycerides, cholesterol esters and phospholipids and also as free fatty acids. The fatty acid composition of triglycerides (measured in blood after an overnight fast) is closely related to dietary intake on a short-term basis and has been used to monitor dietary changes in

subjects following special diets (Moore *et al.*, 1977). A diet rich in polyunsaturated fatty acids causes an increase in linoleic acid in triglycerides within seven days and linoleic acid levels reach a plateau if the same diet is continued over a long period. A better indicator of medium term intake is probably fatty acids linked to cholesterol esters and phospholipids, although some studies indicate that plasma levels are modified in accordance with dietary intake within two weeks (Avons, 1985). Long-term variations in plasma fatty acids studied for twelve months indicated good correlation with dietary intake and an absence of seasonal fluctuations (Reeves *et al.*, 1984).

Fatty acid composition of adipose tissue tends to be a good reflector of the intake of linoleic and linolenic acid, but is a poor indicator of the intake of long-chain polyunsaturated fatty acids (Roshanai and Sanders, 1983). Plasma free fatty acid composition in the fasting state, therefore, could well reflect the linoleic and linolenic acid intakes. Plasma or membrane phospholipids are the favoured lipid fraction for studying changes in the proportions of derived essential fatty acids. However, analyses of different lipid fractions may not be feasible in large samples and determination of composition of total esterified fatty acids has been suggested (Lepage and Roy, 1986). One of the aims of this study was to assess fatty acid intakes accurately. There is little information available on fatty acid intakes of Asian women, most of which were estimated based on 24-hour recall or food frequency questionnaires. The objective of this chapter is to relate the dietary fatty acid intakes to the fatty acid composition of plasma lipids and to compare the different lipid fractions in their ability to reflect the quality of fat in the diet. This chapter compares the composition of total esterified, phospholipid and free fatty acids in plasma in relation to their dietary intakes.

6:2 Results

Table 6.2 gives the mean daily intake of fatty acids as a percentage of total fatty acids in Caucasian omnivores, Indian and Caucasian vegetarians. The vegetarians had significantly lower intakes of saturated fatty acids, but monounsaturated fatty acid intakes were not significantly different. The vegetarian groups also had higher intakes of polyunsaturated fatty acids but the intake of linolenic acid was significantly greater in the Caucasian vegetarians.

TABLE 6:2

Mean daily intakes of fatty acids

	CO (n=22)		IV (n=21)		CV (n=18)	
	Mean	SEM	Mean	SEM	Mean	SEM
<i>Total Fatty Acids (g/day)</i>	76.5 ^a	4.06	57.3 ^b	3.09	68.1	4.60
<i>Saturated Fatty Acids</i>						
g/day	31.4 ^a	2.07	18.5 ^b	1.42	22.6 ^b	1.92
% of total	41.0 ^a	1.41	31.9 ^b	1.53	33.7 ^b	2.38
<i>Monounsaturated Fatty Acids</i>						
g/day	31.9	1.99	23.8	1.33	29.4	2.34
% of total	41.4	1.19	42.0	1.63	43.0	1.64
<i>Polyunsaturated Fatty Acids</i>						
g/day	13.1	1.24	15.1	1.25	16.1	1.77
% of total	17.5 ^a	1.55	26.1 ^b	1.72	23.3 ^b	1.59
<i>Linoleic Acid</i>						
g/day	11.8	1.22	14.2	1.25	14.6	1.69
% of total	15.7 ^a	1.55	24.5 ^b	1.75	21.1 ^b	1.57
<i>Linolenic Acid</i>						
g/day	1.0	0.07	0.9	0.10	1.5	0.17
% of total	1.3 ^a	0.08	1.6 ^a	0.21	2.2 ^b	0.22
<i>Linoleic : Linolenic Ratio</i>	12.7 ^a	1.47	19.4 ^b	2.12	10.7 ^a	1.12
<i>Arachidonic Acid</i>						
g/day	0.13 ^a	0.036	0 ^b	0	0.01 ^b	0.02
% of total	0.16 ^a	0.036	0 ^b	0	0.007 ^b	0.003
<i>Eicosapentaenoic Acid</i>						
g/day	0.08	0.024	-	-	-	-
% of total	0.1	0.03	-	-	-	-
<i>Docosahexaenoic Acid</i>						
g/day	0.10	0.02	-	-	-	-
% of total	0.1	0.03	-	-	-	-

^{a,b}: Values with unlike superscript letters are significantly different from each other.

The fatty acid compositions of total plasma lipids and fractions are summarised in Tables 6:3 to 6:5. Table 6:3 gives the composition of free fatty acids in plasma. Concentrations of monounsaturated fatty acids (16:1 and 18:1) were significantly lower in the Indian women in all plasma fractions. This is in agreement with the levels reported in Gujarati men drawn from the same area of London (Miller *et al.*, 1988). Both the vegetarian groups had considerably higher levels of linoleic acid in all fractions,

TABLE 6:3 Composition of total esterified fatty acids (TEFA) in plasma

Fatty Acids (mg/l)	CO (n=22)				IV (n=20)				CV (n=18)			
	Concentration		Weight (%)		Concentration		Weight (%)		Concentration		Weight (%)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
14:0	30	3.8	0.9	0.08	26	2.9	0.8	0.06	26	3.4	0.8	0.07
16:0	668	45.0	21.3 ^a	0.58	578	31.8	19.0 ^b	0.35	608	35.1	19.2 ^b	0.46
16:1	78 ^a	9.7	2.4 ^a	0.25	40 ^b	4.5	1.3 ^b	0.09	64	9.6	1.9 ^a	0.23
18:0	211	10.9	6.8 ^a	0.15	205	12.7	6.7	0.13	202	10.1	6.4 ^b	0.11
18:1n-9	649	46.4	20.7 ^a	0.81	515	28.1	17.0 ^b	0.41	612	45.6	19.1 ^a	0.75
18:2n-6	821 ^a	48.0	26.7 ^a	1.13	1019 ^b	46.9	34.0 ^b	0.91	992 ^b	50.9	31.9 ^b	1.39
18:3n-3	11	1.2	0.3	0.04	10	1.0	0.3	0.03	12	1.9	0.4	0.29
20:3n-6	35 ^a	2.8	1.1 ^a	0.05	38	3.3	1.3 ^a	0.08	46 ^b	3.3	1.5 ^b	0.08
20:4n-6	174	9.9	5.6	0.25	179	13.3	6.0	0.42	198	10.9	6.4	0.34
20:5n-3	24	2.7	0.8	0.07	17	2.6	0.6	0.09	22	2.2	0.7	0.05
22:6n-3	38 ^a	4.3	1.2 ^a	0.11	20 ^b	2.1	0.6 ^b	0.06	31 ^a	2.8	1.0 ^a	0.08
Total FA (g/l)	3.1	0.17			3.0	0.15			3.2	0.15		

^{a,b}: Values with unlike superscripts are significantly different from each other.

TABLE 6:4 Fatty acid composition of plasma phospholipids (PFA)

Fatty Acids (mg/dl)	CO (n=22)				IV (n=21)				CV (n=18)			
	Concentration		Weight (%)		Concentration		Weight (%)		Concentration		Weight (%)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
14:0	0.6	0.06	0.3	0.03	0.6	0.04	0.3	0.02	0.6	0.04	0.3	0.02
14:1	0.6	0.08	0.30	0.004	0.5	0.04	0.3	0.02	0.6	0.13	0.3	0.07
16:0	61.0 ^a	2.36	30.1	0.83	51.0 ^b	1.52	28.0	0.76	57.8 ^a	2.65	28.0	0.82
16:1	0.6 ^a	0.15	0.3 ^a	0.08	0.1 ^b	0.03	0.1 ^b	0.02	0.4	0.10	0.2	0.04
18:0	28.7	0.93	14.2 ^a	0.30	28.1	1.14	15.3 ^b	0.37	27.2	0.82	13.3 ^c	0.26
18:1n-9	27.5 ^a	1.23	13.5 ^a	0.44	19.7 ^b	0.94	10.7 ^b	0.39	27.6 ^a	1.45	13.4 ^a	0.48
18:2n-6	44.4 ^a	2.87	21.7 ^a	1.13	49.6	2.51	26.9 ^b	0.94	54.9 ^b	3.32	26.5 ^b	1.07
18:3n-3	0.5 ^a	0.06	0.26 ^a	0.03	0.1 ^b	0.01	0.08 ^b	0.007	0.4 ^a	0.05	0.17 ^a	0.020
20:0	0.3	0.02	0.17 ^a	0.01	0.4	0.03	0.21 ^b	0.01	0.4	0.02	0.19	0.01
20:1	0.6	0.07	0.31	0.03	0.5	0.05	0.29	0.02	0.7	0.07	0.33	0.03
20:3n-6	7.9	0.68	3.9	0.36	6.9	0.67	3.8	0.39	6.9	0.58	3.4	0.29
20:4n-6	15.8	1.18	7.7	0.49	16.3	1.11	8.8	0.49	18.5	1.08	9.1	0.54
20:5n-3	2.0 ^a	0.18	0.97 ^a	0.068	0.7 ^b	0.09	0.36 ^b	0.045	1.0 ^b	0.07	0.47 ^b	0.031
22:4	1.0	0.15	0.52	0.08	1.1	0.20	0.60	0.12	1.0	0.14	0.50	0.07
22:5n-6	0.9	0.18	0.48	0.10	0.8	0.18	0.47	0.11	0.7	0.10	0.32	0.05
22:5n-3	1.2 ^a	0.11	0.60	0.05	1.0 ^b	0.09	0.51	0.04	1.3 ^a	0.94	0.66	0.05
22:6n-3	4.6 ^a	0.51	2.26	0.19	2.2 ^b	0.20	1.2	0.09	3.8 ^a	0.36	1.9 ^a	0.20
Total FA (mg/dl)	203.1 ^a	6.62		-	183.3 ^b	5.17		-	205.8 ^a	6.52		-

^{a,b,c} Values with unlike superscript letters are significantly different from each other

TABLE 6:5 **Composition of free fatty acids in plasma (FFA)**

Fatty Acids (mg/dl)	CO (n=22)				IV (n=21)				CV (n=18)			
	Concentration		Weight (%)		Concentration		Weight (%)		Concentration		Weight (%)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
14:0	0.28	0.029	1.6	0.19	0.27	0.024	1.6	0.19	0.31	0.046	1.5	0.16
16:0	4.37	0.312	24.5	0.70	4.16	0.239	23.9	0.83	5.15	0.449	23.8	0.86
16:1	0.70 ^a	0.099	3.8 ^a	0.39	0.42 ^a	0.077	2.4 ^a	0.40	0.70 ^a	0.077	3.2	0.26
18:0	3.55	0.340	19.6	1.06	3.20	0.212	18.4	0.89	4.12	0.459	18.6	1.27
18:1n-9	5.89	0.680	30.9	1.25	5.21	0.487	28.4 ^a	0.96	7.12	0.830	32.2 ^a	1.42
18:2n-6	2.06 ^a	0.199	11.4 ^a	0.56	3.59 ^a	0.345	19.6 ^a	0.90	3.30 ^a	0.243	15.8 ^a	0.85
18:3n-3	0.13	0.013	0.7	0.07	0.20	0.049	1.0	0.91	0.21	0.027	1.0	0.13
20:3n-6	0.08	0.025	0.5 ^a	0.14	0.05	0.005	0.3	0.03	0.03	0.005	0.2 ^a	0.02
20:4n-6	0.28 ^a	0.038	1.5 ^a	0.17	0.25	0.020	1.4 ^a	0.11	0.19 ^a	0.020	0.9 ^a	0.06
20:5n-3	0.05 ^a	0.008	0.3 ^a	0.04	0.01 ^b	0.003	0.07 ^a	0.017	0.01 ^b	0.002	0.07 ^a	0.007
22:6n-3	0.113 ^a	0.015	0.6 ^a	0.07	0.05 ^b	0.009	0.2	0.04	0.05 ^b	0.009	0.3	0.05
Total FA (mg/dl)	18.4	1.55			17.8	1.24			21.8	1.87		

^{a,b,c}: Values with unlike superscript letters are significantly different from each other.

which appears to be directly related to their linoleic acid intake. However, linolenic acid (18:3) levels were not significantly different in total plasma fatty acids and in free fatty acids, but there was a significant difference in the concentrations of linolenate in plasma phospholipids. Indians had the lowest levels of linolenate compared to Caucasians. Arachidonate (20:4n-6) levels were higher in the Caucasian vegetarians in plasma phospholipids as well as in total plasma, but did not achieve statistical significance. In contrast, the amount of arachidonate in the free fatty acid fraction of the plasma was significantly lower in Caucasian vegetarians compared to other groups. EPA levels are significantly lower in plasma phospholipids and free fatty acids of the vegetarian groups which could indicate lack of EPA in their diets. Concentration of DHA in plasma free fatty acids was significantly lower in both groups of vegetarians but, unlike Caucasian vegetarians, in the Indians the proportion of DHA continued to be significantly lower in total plasma and also in plasma phospholipids. Although the proportion of DHA was lower in Caucasian vegetarians compared to the omnivores, the differences were not statistically significant. The total amounts of fatty acids in plasma and total free fatty acids were not statistically different in these groups. However the Indians had significantly lower levels of total fatty acids in plasma phospholipids. This may be related to the lower levels of HDL-cholesterol found consistently in the Indians and a significant correlation ($r=0.27$, $p=0.02$) was found between total phospholipid fatty acids and HDL concentrations in this study.

Table 6:6 shows the correlation of dietary fatty acid intake with fatty acid levels in plasma, plasma phospholipids and plasma free fatty acids. Saturated fatty acid intake, both as grams per day and as a proportion of energy, did not correlate significantly with saturated fatty acid levels in total plasma or in plasma free fatty acids. However, the proportion of energy from saturated fat significantly correlated with the concentration of total saturated fatty acids in plasma phospholipids. A significant correlation was also found between the proportion of monounsaturated fatty acids in the plasma phospholipid fraction and the daily intake of monounsaturated fatty acids, but these should be interpreted cautiously because plasma levels of these fatty acids in the body may not reflect their dietary intakes owing to the body's capacity to metabolize these fatty acids through desaturation and elongation.

TABLE 6:6

Correlation of dietary fatty acids (intake per day) with plasma fatty acids. r (p value)

	Total Esterified FA		Phospholipid FA		Free FA	
	Conc.	Wt (%)	Conc.	Wt (%)	Conc.	Wt (%)
<u>Total Saturates</u> g/day	0.064 (0.320)	0.084 (0.271)	0.195 (0.075)	-0.011 (0.467)	-0.010 (0.470)	0.076 (0.289)
% energy	0.135 (0.161)	0.141 (0.150)	0.294 (0.014)	0.128 (0.174)	-0.019 (0.445)	0.113 (0.204)
<u>Total Monounsaturates</u> g/day	0.141 (0.150)	0.208 (0.062)	0.178 (0.095)	0.216 (0.055)	0.0320 (0.407)	0.137 (0.157)
% energy	0.173 (0.102)	0.196 (0.074)	0.022 (0.435)	0.049 (0.360)	-0.091 (0.253)	0.059 (0.332)
<u>Total Polyunsaturates</u> g/day	0.020 (0.443)	0.215 (0.056)	0.134 (0.163)	0.233 (0.042)	0.086 (0.265)	0.154 (0.129)
% energy	0.019 (0.446)	0.194 (0.077)	0.088 (0.260)	0.226 (0.047)	0.230 (0.044)	0.394 (0.001)
<u>Linoleic Acid (18:2n-6)</u> g/day	0.073 (0.297)	0.258 (0.028)	0.264 (0.025)	0.375 (0.002)	0.1452 (0.143)	0.251 (0.031)
% energy	0.099 (0.234)	0.277 (0.020)	0.260 (0.026)	0.416 (0.001)	0.288 (0.016)	0.466 (0.000)
<u>Linoleic Acid vs EPA</u> g/day	-0.085 (0.268)	-0.049 (0.382)	-0.176 (0.097)	-0.224 (0.049)	-0.288 (0.016)	-0.235 (0.041)
% energy	-0.079 (0.281)	-0.036 (0.397)	-0.320 (0.008)	-0.384 (0.002)	-0.366 (0.003)	-0.292 (0.014)
<u>Linoleic Acid vs DHA</u> g/day	-0.226 (0.047)	-0.221 (0.051)	-0.132 (0.165)	-0.155 (0.128)	-0.261 (0.026)	-0.248 (0.032)
% energy	-0.357 (0.003)	-0.382 (0.002)	0.287 (0.016)	-0.320 (0.008)	-0.296 (0.014)	-0.262 (0.026)
<u>Linoleic : Linolenic</u> vs EPA	-0.042 (0.380)	0.032 (0.409)	-0.274 (0.020)	-0.339 (0.005)	-0.218 (0.053)	-0.166 (0.111)
<u>Linoleic : Linolenic</u> vs DHA	-0.230 (0.044)	-0.268 (0.023)	-0.172 (0.103)	-0.202 (0.067)	-0.134 (0.162)	-0.166 (0.111)

TABLE 6:6 (continued)

Correlation of dietary fatty acids (intake per day) with plasma fatty acids. r (p value)

	Total Esterified FA		Phospholipid FA		Free FA	
	Conc.	Wt (%)	Conc.	Wt (%)	Conc.	Wt (%)
<u>Linolenic Acid (18:3n-3)</u> g/day	0.101 (0.230)	0.137 (0.158)	0.161 (0.118)	0.171 (0.104)	0.230 (0.044)	0.291 (0.015)
% energy	0.068 (0.308)	0.111 (0.208)	0.143 (0.146)	0.157 (0.124)	0.297 (0.013)	0.382 (0.002)
<u>Eicosapentaenoic Acid (20:5n-3)</u> g/day	0.099 (0.235)	0.062 (0.326)	0.492 (0.000)	0.397 (0.001)	0.265 (0.024)	0.295 (0.014)
% energy	0.105 (0.220)	0.065 (0.315)	0.513 (0.000)	0.412 (0.001)	0.285 (0.017)	0.323 (0.008)
<u>Docosahexaenoic Acid (22:6n-3)</u> g/day	0.240 (0.038)	0.326 (0.007)	0.326 (0.007)	0.302 (0.012)	0.472 (0.000)	0.361 (0.003)
% energy	0.301 (0.012)	0.336 (0.006)	0.322 (0.008)	0.299 (0.013)	0.472 (0.000)	0.384 (0.002)

Dietary intake of total polyunsaturated fatty acids appears to be better reflected in plasma fatty acids. Daily intake (g/day) correlated significantly with the proportion of total polyunsaturates in plasma as well as in plasma phospholipids. However, the proportion of energy contributed by polyunsaturates correlated well with the concentration and proportion of polyunsaturates in plasma free fatty acids. There was a strong relationship (Figures 6:2a,b and 6:3a,b) between linoleic acid intake and linoleic acid concentrations in plasma, plasma phospholipids and plasma free fatty acids. Linolenic acid intakes correlated with linolenic acid levels in ^{the} free fatty acid fraction but not with levels in plasma and plasma phospholipids. Highly significant correlations were also found between intake of DHA and its concentration in all fractions of plasma. Intake of EPA correlated significantly with EPA levels in both plasma phospholipids and plasma free fatty acids, but not with levels in total fatty acids in plasma.

Figure 6:2a Relationship of dietary and total esterified linoleate in plasma

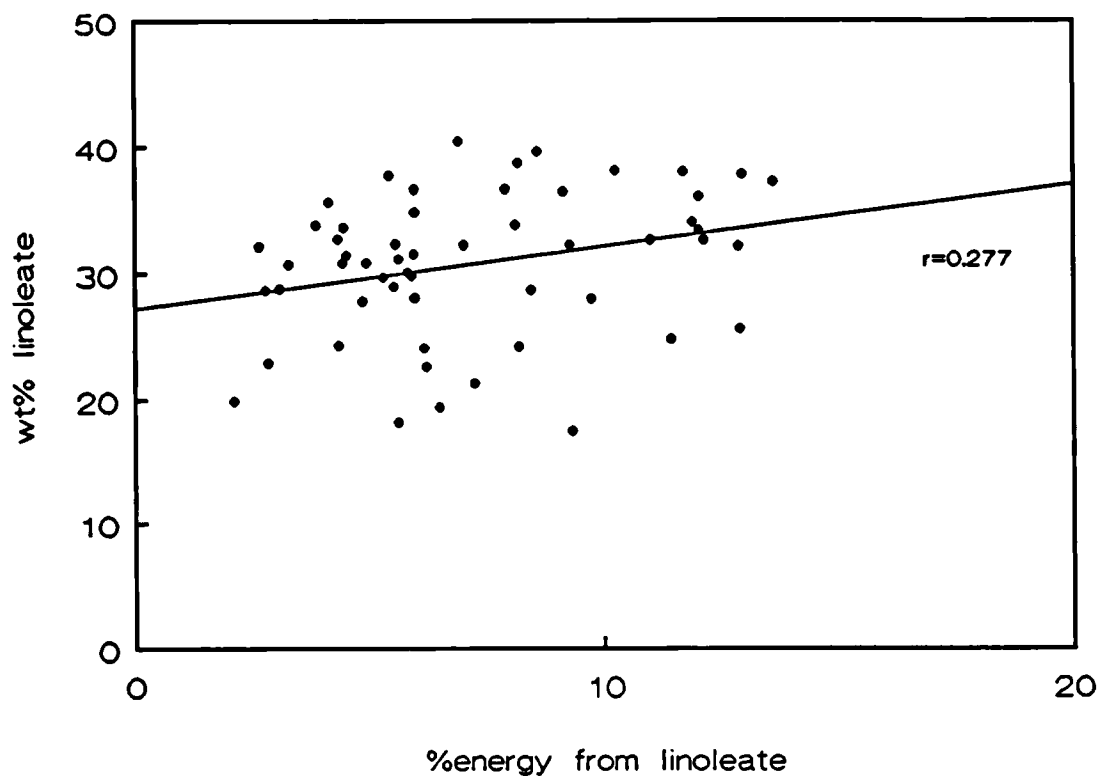


Figure 6:2b Relationship of dietary and free linoleate in plasma

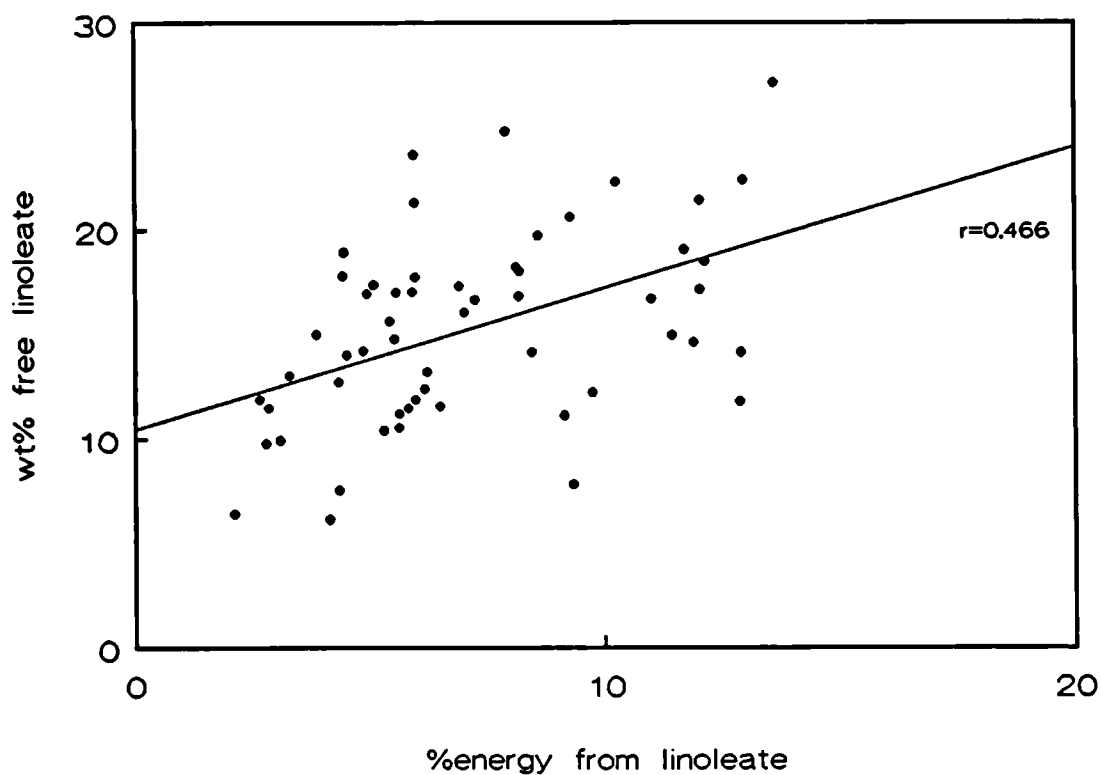


Figure 6:3a Relationship of dietary and plasma phospholipid linoleate

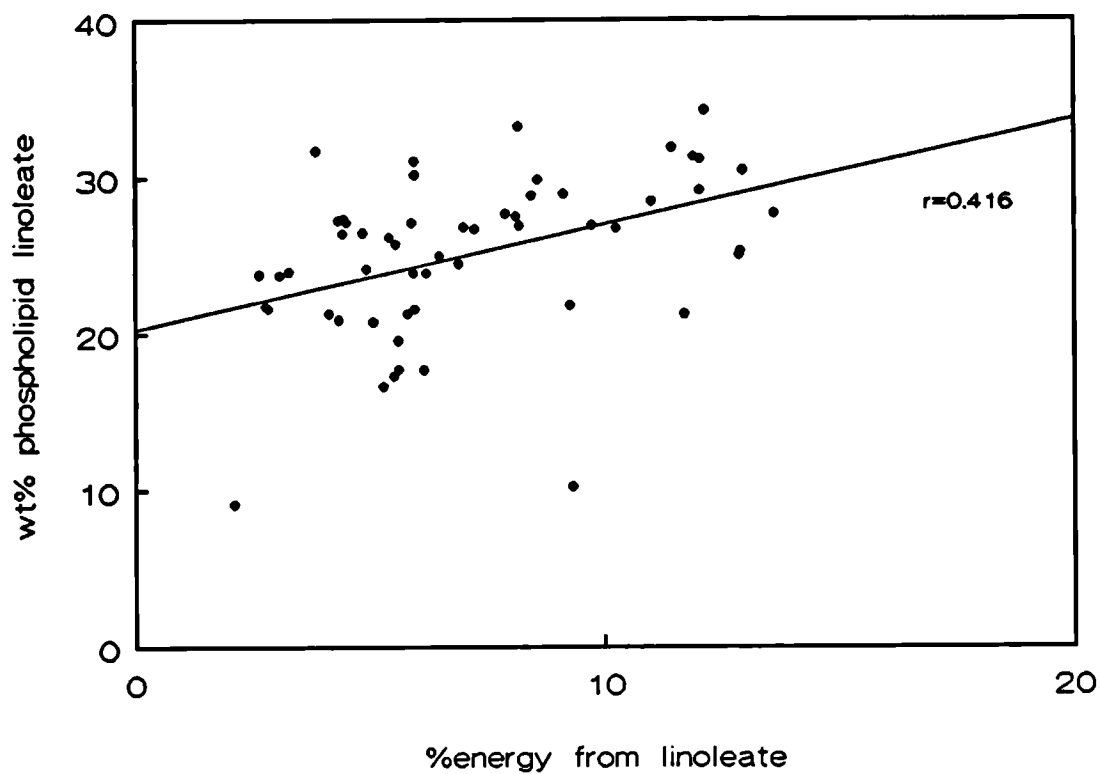
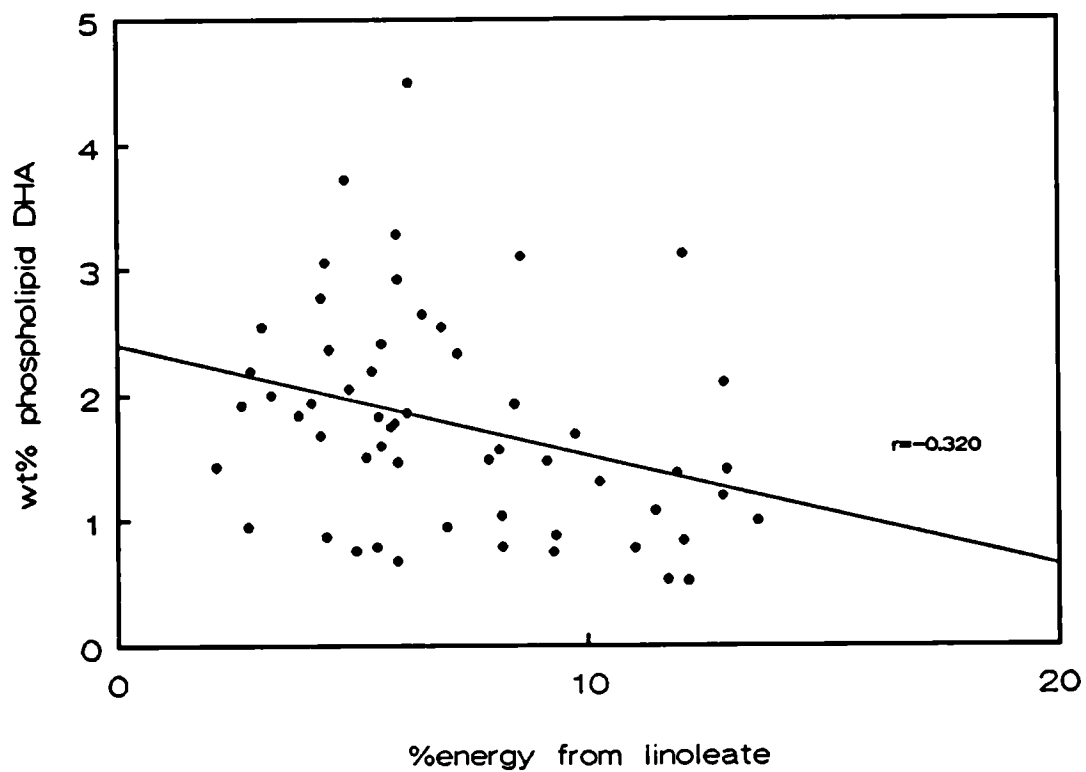


Figure 6:3b Relationship of dietary linoleate and phospholipid DHA



The strong correlations between dietary EPA and DHA and plasma levels could be related to the skewed distribution of intakes in the subjects due to the inclusion of vegetarians in the analyses. The levels of both EPA and DHA in all fractions of plasma were negatively correlated with linoleic acid intakes, which may mean that in the absence of n-3 fatty acids, linoleic acid and its derivatives are predominant in phospholipids (Figure 6:3b) as well as in the adipose tissue. Negative correlation was found between the ratio of linoleic to linolenic acids and the concentrations of EPA and DHA. It is known that linolenic acid is preferred as a substrate for β -oxidation in the presence of high intakes of linoleic acid, which could also lead to higher amounts of n-6 fatty acids and their derivatives in the body and phospholipid arachidonate levels were higher in the vegetarians, but not significantly so.

6:3 Discussion

One of the aims of this study was to accurately assess the quality, as well as the quantity, of dietary fat in these subjects. Most studies that reported individual fatty acid intakes either used food frequency questionnaires (Lopes *et al.*, 1991) or household inventory method (McKeigue *et al.*, 1988) to assess fatty acid intakes, which may well have given inaccurate estimates. Some have assessed fatty acid intakes over a three-day or a five-day period. In this study dietary intakes were recorded over a period of seven days, which included the weekend days when diet is known to differ. Considerable effort was made to assess the actual fatty acid intakes by collecting and analyzing cooking fats and spreading fats used in the individual households. Additionally, commercially prepared foods consumed by the subjects were analyzed for their fatty acid composition. Dietary intakes of individual subjects were calculated only after incorporating their specific dietary fatty acid composition data to ensure accuracy in estimating individual fatty acid intakes.

The results of the study show that in general fatty acid composition of plasma lipids is a reliable index of polyunsaturated fatty acid intake but not of saturated or monounsaturated fatty acids. Linoleic acid intakes, both as grams per day and as percentage of energy, correlated strongly with linoleic acid concentrations in plasma phospholipids and free fatty acids. The proportion of linoleic acid in the diet being greater than any other polyunsaturated acids would have led to its incorporation in greater amounts into adipose tissue which would be reflected in the free fatty acid fraction of

plasma lipids in a fasting state. Linoleic acid is also incorporated into plasma phospholipids along with its derivatives in preference to linolenic acid. Therefore plasma phospholipids appear to be better markers of linoleic acid. However, linolenic acid levels in the free fatty acid fraction of plasma could be indicative of linolenic acid incorporated into depot fat and may be assumed as a marker of its intake in the past. Fatty acid composition of plasma phospholipid is a reliable indicator of medium-term linoleic acid intake but not of linolenic acid, whereas the composition of free fatty acids in a fasting state gives a reliable indication of the intake of both linoleic and linolenic acid in the past.

EPA and DHA intakes correlated significantly with their levels in plasma phospholipids as well as in the free fatty acid fraction, but it is known that long-chain fatty acids are preferably incorporated into plasma phospholipids rather than into adipose tissue. Therefore, the intake of EPA and DHA can be assessed reliably by their concentrations in plasma phospholipids.

A negative correlation was found between the ratio of linoleic and linolenic acid intakes and the levels of EPA and DHA in all plasma lipid fractions, indicating a preferential incorporation of n-6 fatty acids. The balance of n-6 and n-3 fatty acids is of importance in the context of the role of their long-chain derivatives in phospholipids. Eicosanoids derived from n-6 fatty acids are pro-aggregatory, promoting thrombogenesis. This pro-thrombotic tendency of n-6 fatty acid derivatives may be of concern, particularly with respect to their role in atherosclerosis. Eicosanoids and docosanoids derived from n-3 fatty acids have been shown to be not only weakly active but also anti-thrombogenic. Several intervention trials using fish oil supplements have reported prolonged bleeding time (Sanders *et al.*, 1981).

In view of the fact that Indians have been shown to be susceptible to coronary heart disease, the balance of n-6 and n-3 fatty acids in their diet may be important. The Indians had significantly lower levels of DHA in plasma, especially in phospholipid and total lipid fractions, which may have been a consequence of the high ratio of linoleic to linolenic acid in their diet. In contrast, linolenic acid intakes were higher in Caucasian vegetarians and could explain their plasma levels of DHA, which were almost comparable to those of omnivores. However, omnivores in this study had lower levels of DHA (2%)

compared to other studies which reported higher DHA levels (4%) in plasma phospholipids of male subjects determined in the same laboratory (Sanders *et al.*, 1978; Miller *et al.*, 1988; Sanders *et al.*, 1989).

In conclusion, plasma phospholipids are good indicators of linoleic acid, EPA and DHA, whereas the free fatty acid fraction of plasma in a fasting state is a reliable index of the past intake of linoleic and linolenic acids. Concentrations of DHA in plasma phospholipids may be good predictors of the linoleic : linolenic acid ratio of the diet, particularly in the vegetarians. It can be concluded that measuring fatty acid composition of plasma lipids can be successfully used to assess the quality of dietary fat in individuals. This may be a relatively easy way of evaluating compliance in an individual during dietary intervention, because a change in quality of fat invariably means an increase in the intake of polyunsaturated fatty acids.

6:4 Summary

1. Fatty acid intakes in Caucasian omnivores, Indian vegetarians and Caucasian vegetarians were compared to fatty acid composition of total esterified fatty acids, phospholipids and free fatty acids in plasma.
2. The Indians had significantly lower levels of total fatty acids in plasma phospholipids. Concentrations of linoleic acid were greater in vegetarians in all plasma fractions. Indian vegetarians also had the lowest levels of linolenic acid in plasma phospholipids compared to both groups of Caucasians. EPA and DHA concentrations were lower in the vegetarians but the Indians had a significantly lower proportion of DHA in total plasma lipids and in plasma phospholipids.
3. Total polyunsaturated fatty acids correlated significantly with their total concentrations in plasma and plasma phospholipids. There was a strong relationship between intakes of linoleic acid and DHA and their concentrations in all plasma fractions. EPA intakes in these subjects correlated significantly with plasma phospholipids and free fatty acid fractions. A negative correlation was also found between the ratio of 18:2n-6 and 18:3n-3 intakes and the levels of EPA and DHA in all plasma lipid fractions, indicating a preferential incorporation of n-6 fatty acids.
4. It is concluded that plasma phospholipids are good indicators of 18:2n-6, EPA and DHA intakes, whereas the free fatty acid fraction of plasma in a fasting state is a reliable index of past intake of 18:2n-6 and 18:3n-3. The ratio of 18:2n-6 to 18:3n-3 in the diet may be a good predictor of DHA in plasma phospholipids.

CHAPTER 7

RETINOL, α -TOCOPHEROL AND LIPOPROTEIN CONCENTRATIONS IN PLASMA

7:1 Introduction

The role of plasma lipoproteins in the development of atherosclerosis is well known. Their relative concentrations in plasma are regarded as good predictors of coronary atherosclerosis. Diet, especially dietary fat, seems to influence the concentrations and metabolism of plasma lipoproteins in the body (Section 2, Chapter I). Although one of the main functions of plasma lipoproteins is the transport of lipids in the aqueous medium in the body, they have a vital role as carriers of fat-soluble vitamins like vitamin A or retinol, and vitamin E or tocopherol.

7:1:1 Vitamin A

Vitamin A comprises a group of compounds: retinol, retinaldehyde and retinoic acid, collectively called retinoids. Vitamin A is essential for vision, growth, cellular differentiation and proliferation, reproduction and the integrity of the immune system. The body's need for vitamin A can be met by preformed retinoids, usually in animal products, or by consumption of carotenoid precursors of vitamin A such as β -carotene, α -carotene and cryptoxanthin formed by plants and may also be present in some animal fats, notably dairy fat. Both retinoids and carotenoids vary qualitatively as well as quantitatively in vitamin A activity due to the strict structural requirements essential for the biological activity of vitamin A. Out of more than 500 carotenoids only 50 are precursors of retinol and all-*trans*- β -carotene is the most active on a weight basis and makes the most important quantitative contribution to vitamin A intake in man. The bio-availability of carotenoids in many foods is not as efficient as that of retinol, therefore a system of retinol equivalents was defined.

Six micrograms of dietary β -carotene is equivalent to 1 μg of retinol. The vitamin A activity in foods is currently expressed as retinol equivalents (RE) where 1 RE is defined as 1 μg of all-*trans*-retinol, 6 μg of all-*trans*- β -carotene or 12 μg of other provitamin A carotenoids. As the absorption of β -carotene from milk fat is complete, 2 μg of β -carotene from dairy sources \equiv 1 RE. The richest sources of preformed retinol are liver, fish liver oils and appreciable amounts are also present in eggs and butter fat. Carrots and dark green leafy vegetables are rich sources of biologically-active carotenoids, especially β -carotene. The UK RNI for vitamin A is 600 μg or RE for women.

Vitamin A deficiency is found most commonly in children under 5 years of age and is usually due to insufficient dietary intake. Deficiency may also occur in chronic fat malabsorption. Prominent clinical signs are ocular and range in increasing severity from nightblindness and conjunctival xerosis to corneal xerosis or xerophthalmia which could be irreversible if left untreated. Other less specific deficiency symptoms include loss of appetite, hyperkeratosis, increased susceptibility to infections and metaplasia and keratinization of epithelial cells of the respiratory tract and other organs. Although rare in the developed world, vitamin A deficiency is a major nutritional problem in developing countries (FAO, 1988). The absorption and utilization of carotenoids and vitamin A are enhanced by dietary fat protein and vitamin E. In the fasting state vitamin A is transported by proalbumin and retinol binding protein. Deficiencies of a variety of other nutrients including protein, α -tocopherol, iron and zinc are known to adversely affect vitamin A transport, storage and utilization (Underwood, 1984). Retinoids have been known to retard experimentally produced cancers at different sites such as skin, bladder and breast in animal models. Several studies have shown an association between a low concentration of retinol in plasma with cancer, especially lung cancer, but the studies are by no means conclusive (Pastorino *et al.*, 1987; NRC, 1989). Most carotenoids, unlike retinol, trap free radicals (Burton and Ingold, 1984) which can cause neoplastic changes in cells. The anti-cancer effects of carotenoids may be related to their unique anti-oxidant properties rather than to their provitamin A activity. Zeigler *et al.* (1986), in an epidemiological study, correlated the dietary intake of carotenoid-rich vegetables with a lowered risk of cancer in Caucasians. In the present study carotenoid intake was not estimated but since primary food sources of β -carotene are few, their consumption could be assumed to be good indicators of carotene intake.

7:1:2 Vitamin E

Two groups of compounds found in plants have varying vitamin E biological activity: tocopherols and tocotrienols. Tocopherols are the most active form of vitamin E, especially α -tocopherols, while β -, γ - and δ -tocopherols have variable degrees of activity. For dietary purposes vitamin E activity is expressed as tocopherol equivalents (TE), where 1 TE is equal to 1 mg of α -tocopherol. β - and γ -tocopherols have only 50 and 30% of the activity of α -tocopherol. Tocopherols are known chemically as antioxidants and prevent the oxidation of unsaturated fatty acids by trapping peroxy free radicals. In animal tissues α -tocopherol is found associated with polyunsaturated fatty

acids in membrane phospholipids. In vitamin E deficiency, the oxidation of PUFA is more readily propagated along the membrane, leading to cell damage. The antioxidant function of vitamin E is also aided by vitamin C and selenium as a component of glutathione peroxidase. Vitamin E deficiency is rare and occurs only in premature infants in whom deficiency has been associated with other clinical disorders and in patients who do not absorb fat normally. The primary signs of deficiency in animals are reproductive failure, muscular dystrophy and neurological abnormalities.

Absorption of α -tocopherol is relatively inefficient, ranging from 20 to 80%. Normal bile secretion and pancreatic function are essential for tocopherol absorption. Tocopherol is secreted into the lymph in chylomicrons, taken up into the liver with chylomicron remnants, and subsequently secreted into the blood in VLDL (Traber *et al.*, 1988). As VLDLs are metabolized, tocopherol is transferred to LDLs and HDLs. In women, HDLs appear to carry more tocopherol than does LDL (Behrens *et al.*, 1982). Blood concentrations of total tocopherols in normal adults range from 0.5 to 1.2 mg/dl. Because α -tocopherol is carried by lipoproteins, the plasma lipid content can influence the tocopherol concentration, therefore vitamin E expressed as the ratio of the sum of plasma cholesterol and triglycerides has been suggested (Thurnham *et al.*, 1986). When plasma vitamin E levels are considerably below normal, red cells become susceptible to excessive haemolysis (Leonard and Losowsky, 1971).

The tocopherol content of foods varies greatly depending on processing, storage and preparation procedures, during which large losses may occur. The richest sources are common vegetable oils and products made from them such as margarine. Wheat germ, nuts and whole grains are good sources and green vegetables supply appreciable amounts of this nutrient. The vitamin E content of the diet varies widely depending on the type and amount of fat present and losses that may occur during pregnancy and cooking. Vitamin E intakes in U.K. adults have been reported to be 6-9 mg/day.

There appears to be a relationship between vitamin E and PUFA intakes. The vitamin E requirement in animals increases when PUFA intakes increase and this may be applicable to humans too. The consumption of fish oils which contain highly unsaturated fatty acids but are relatively low in vitamin E may disturb their balance in the membranes. A PUFA to vitamin E ratio of 0.4 in the diet has been suggested as

adequate for humans, but this needs further confirmation as these figures were derived using linoleic acid rather than linolenic, eicosapentaenoic and docosahexaenoic acids. There is no recommended allowance set in the UK for vitamin E but the US recommendations are 10 mg of α -TE per day for men and 7 mg per day for women. PUFA intakes in a vegetarian diet are usually high and indeed both groups of vegetarians had significantly higher intakes of PUFA. Additionally, in its role as an antioxidant vitamin E may also influence the modification of LDL through oxidation (Esterbauer *et al.*, 1991) and thereby influence atherogenesis.

This chapter compares the plasma concentrations of α -tocopherol, retinol and lipoproteins in pre-menopausal Caucasian omnivores, Indian and Caucasian vegetarians and explores the relationship between diet and these variables in plasma.

7:2 Methods

Analytical methods are described in Chapter 2. Between group comparisons were made by ANOVA and Duncan's multiple range test. Relationships between variables were assessed by both univariate and multivariate analyses.

7:3 Results

7:4:1 Retinol and Tocopherol Concentrations in Plasma

Retinol concentrations in Indian women were significantly lower than in both groups of Caucasians (Table 7:1).

The daily source of retinol for the vegetarians was mainly in the form of carotenoids. Plasma retinol levels tend not to reflect dietary intake unless stores are grossly depleted. The lower plasma concentrations of retinol in the Indians are due to lower levels of carrier protein. It is to be noted that SHBG levels were lower in this group.

Tocopherol concentrations were significantly greater in Caucasian vegetarians compared to other groups in this study, and similar to the levels reported in British women. Differences in plasma tocopherol persisted when tocopherol levels were corrected for plasma cholesterol concentrations. There was also a significant correlation between dietary intake of tocopherol and plasma concentrations. Caucasian vegetarians

had higher intakes of wholefoods which provided a considerable proportion of tocopherol in their diet. In contrast, the lower intakes of vitamin E correspond to significantly lower levels found in Caucasian omnivores and Indian vegetarians.

TABLE 7:1

Plasma retinol and tocopherol concentrations

	CO (n=22)		IV (n=22)		CV (n=18)		British Women* 25-34 yrs
	Mean	SEM	Mean	SEM	Mean	SEM	
<i>Retinol</i> ($\mu\text{mol/l}$)	2.96 ^a	0.197	2.24 ^b	0.082	2.74 ^a	0.141	1.8
<i>α-Tocopherol</i> ($\mu\text{mol/l}$)	12.8 ^a	1.37	12.7 ^a	1.24	20.0 ^b	1.05	22.7
<i>Tocopherol:Cholesterol Ratio</i>	2.5 ^a	0.26	2.7 ^a	0.24	4.5 ^b	0.177	4.51
<i>Correlation Coefficient Plasma Tocopherol vs Dietary Tocopherol</i>	$r = 0.2952 \quad p = 0.014$						
<i>Tocopherol:Cholesterol Ratio vs Dietary Tocopherol</i>	$r = 0.4132 \quad p = 0.001$						

* Gregory *et al.* (1990).

^{a,b}: Values with unlike superscript letters are significantly different.

7:4:2 Plasma Lipoprotein Concentrations

Table 7:2 summarizes plasma lipid concentrations of the groups. Triglyceride concentrations were higher in Indian women compared to the Caucasians, but they were not statistically significant. Total cholesterol levels were significantly greater in Caucasian omnivores compared to Caucasian vegetarians. The Indian vegetarians had intermediate levels which were not statistically significant. Total HDL and HDL fractions were all greater in Caucasian vegetarians. Total HDL levels were significantly higher compared to Indian vegetarians, while HDL₂ concentrations were significantly greater compared to Caucasian omnivores and Indian vegetarians. However, there was no statistically significant difference in HDL₃ cholesterol levels. HDL cholesterol, particularly the HDL₂ fraction, is believed to be protective against coronary heart disease.

Caucasian omnivores had significantly greater levels of LDL-cholesterol compared to Caucasian vegetarians, but not Indian vegetarians. However, Caucasian vegetarians had the lowest levels of LDL-cholesterol. ApoB, which is associated with LDL in plasma, was also significantly lower in Caucasian vegetarians compared to both omnivores and Indians. Higher levels of ApoB are considered to be one of the predisposing risk factors for coronary heart disease. ApoA_I was also significantly lower in Caucasian vegetarians and so was the ratio of ApoB : ApoA_I. Apolipoprotein(a), which indicates the levels of Lp(a) were higher in the Indians compared to Caucasians but due to the large variation in concentrations in the Indian vegetarians, the untransformed differences between groups were not statistically significant. However, when the data was log transformed the Apo(a) levels in Indians were significantly greater compared to Caucasian omnivores and vegetarians. Geometric means and 95% confidence limits clearly show the different distribution of Apo(a) concentration in Indians compared to both groups of Caucasians.

TABLE 7:2

Plasma lipoprotein concentrations

Lipids	CO (n=22)		IV (n=22)		CV (n=18)		British Women 25-34 yrs
	Mean	SE	Mean	SE	Mean	SE	
<i>Triglycerides (mg/dl)</i>	87	9.7	94	7.4	71	6.2	-
<i>Total Cholesterol (mmol/l)</i>	5.19 ^a	0.212	4.78	0.183	4.44 ^b	0.150	5.1
<i>HDL-Cholesterol (mmol/l)</i>	1.66	0.072	1.47 ^a	0.058	1.83 ^b	0.109	1.4
<i>HDL2-Cholesterol (mmol/l)</i>	0.33 ^a	0.035	0.28 ^a	0.030	0.46 ^b	0.060	-
<i>HDL3-Cholesterol (mmol/l)</i>	1.33	0.057	1.22	0.057	1.39	0.066	-
<i>LDL-Cholesterol (mmol/l)</i>	3.07 ^a	0.222	2.76	0.155	2.25 ^b	0.155	-
<i>Apo A1 (g/l)</i>	1.50 ^a	0.085	1.42	0.074	1.25 ^b	0.074	-
<i>Apo B (g/l)</i>	0.88 ^a	0.071	0.83 ^a	0.057	0.55 ^b	0.023	-
<i>ApoB : ApoA1</i>	0.58 ^a	0.028	0.59 ^a	0.028	0.46 ^b	0.024	-
<i>Apo(a) (u/l)</i>	129 ^b	26.3	326 ^a	125.6	125 ^b	31.1	-
<i>Geometric Mean</i>	84		161		79		-
<i>95% Confidence Intervals</i>	55 - 130		94 - 275		49 - 128		-

a,b Values with unlike superscript letters are significantly different.

The results of this study show that all the known risk factors for coronary heart disease such as high levels of cholesterol, LDL, ApoB and Apo(a) were lower in the Caucasian vegetarians. Although the Indians in this study were vegetarians, they differed from the Caucasian vegetarians in that they appear to have higher levels of LDL, ApoB and Lp(a) associated with lower levels of HDL and HDL₂ cholesterol.

7:4:3 The Relationship of Plasma Lipoproteins to Anthropometric and Dietary Variables

To examine the relationship between plasma lipids and anthropometric and dietary variables, univariate and multivariate analyses were undertaken. Tables 7:3 and 7:4 give the correlation coefficients and the probability values.

Triglycerides

Triglyceride concentrations in the subjects did not correlate with any of the dietary variables listed but several significant relationships were found with anthropometric variables. There appears to be a significant effect of body fat on triglyceride levels in plasma. All measurements of skinfold thickness, with the exception of suprailiac, showed significant correlation with triglyceride levels. However, the waist/hip ratio had little effect on triglyceride levels which do not seem to be significantly affected by increasing age. There was a weak relationship between the dietary variables and triglyceride concentrations.

When all the variables that showed significant correlations were included in the multiple regression model, skinfold thickness at biceps was a significant predictor of triglyceride levels in these subjects (Table 7:5). These analyses indicate that obesity is an important factor, particularly upper body obesity, since highly significant correlations were found between upper body skinfold thickness but not with waist/hip ratio which is an index of abdominal obesity.

The Indian vegetarian women in this study had significantly greater measurements of triceps, subscapular and suprailiac measurements compared to other groups, indicating a higher prevalence of upper body obesity. They also had higher triglyceride concentrations, but the differences between groups were not statistically significant. The significant relationship between triglycerides and obesity or body mass index in premenopausal women has also been reported by Wing *et al.* (1989).

TABLE 7:3 Correlation of plasma lipids with anthropometric variables (r, p value) in all subjects

	Triglycerides	Total cholesterol	HDL-cholesterol	LDL-cholesterol	ApoA1	ApoB	Apo(a)
<i>Age</i>	0.0627 <i>p</i> =0.314	0.3541 <i>p</i> =0.002	-0.1331 <i>p</i> =0.157	0.3886 <i>p</i> =0.001	0.1441 <i>p</i> =0.132	0.2344 <i>p</i> =0.033	0.1599 <i>p</i> =0.111
<i>BMI</i>	0.3255 <i>p</i> =0.005	0.2250 <i>p</i> =0.039	-0.4919 <i>p</i> =0.000	0.3050 <i>p</i> =0.009	-0.0180 <i>p</i> =0.445	0.2473 <i>p</i> =0.026	0.1633 <i>p</i> =0.106
<i>Biceps</i>	0.3597 <i>p</i> =0.002	0.2425 <i>p</i> =0.029	-0.4734 <i>p</i> =0.000	0.3513 <i>p</i> =0.007	-0.0432 <i>p</i> =0.370	0.3163 <i>p</i> =0.006	0.0435 <i>p</i> =0.371
<i>Triceps</i>	0.2493 <i>p</i> =0.025	0.2064 <i>p</i> =0.054	-0.3599 <i>p</i> =0.003	0.2639 <i>p</i> =0.022	0.0454 <i>p</i> =0.363	0.2637 <i>p</i> =0.019	0.1234 <i>p</i> =0.174
<i>Subscapular</i>	0.2967 <i>p</i> =0.010	0.3143 <i>p</i> =0.006	-0.5144 <i>p</i> =0.000	0.4474 <i>p</i> =0.000	0.0354 <i>p</i> =0.393	0.3118 <i>p</i> =0.007	0.3303 <i>p</i> =0.005
<i>Suprailiac</i>	0.1590 <i>p</i> =0.109	0.0946 <i>p</i> =0.232	-0.4904 <i>p</i> =0.000	0.2315 <i>p</i> =0.039	-0.0405 <i>p</i> =0.377	0.1371 <i>p</i> =0.144	0.3761 <i>p</i> =0.002
<i>Sum of Skinfolds</i>	0.3097 <i>p</i> =0.013	0.2313 <i>p</i> =0.035	-0.5472 <i>p</i> =0.000	0.3640 <i>p</i> =0.002	-0.0018 <i>p</i> =0.495	0.2759 <i>p</i> =0.015	0.3097 <i>p</i> =0.008
<i>W/H Ratio</i>	0.1515 <i>p</i> =0.120	0.0402 <i>p</i> =0.378	-0.2249 <i>p</i> =0.043	0.1359 <i>p</i> =0.152	-0.1662 <i>p</i> =0.098	0.0432 <i>p</i> =0.369	-0.1870 <i>p</i> =0.076
<i>% Body Fat</i>	0.2952 <i>p</i> =0.010	0.2404 <i>p</i> =0.030	-0.5777 <i>p</i> =0.000	0.3930 <i>p</i> =0.001	0.0290 <i>p</i> =0.411	0.3183 <i>p</i> =0.006	0.2529 <i>p</i> =0.026

TABLE 7:4 Correlation of plasma lipids with dietary variables (r, p value)

Dietary Variables	Triglycerides	Total cholesterol	HDL-cholesterol	LDL-cholesterol	ApoA1	ApoB	Apo(a)
Energy	-0.738 p=0.293	-0.0837 p=0.268	0.1527 p=0.133	-0.1695 p=0.108	-0.0826 p=0.271	-0.1202 p=0.187	-0.1925 p=0.078
% Energy - Protein	0.0186 p=0.445	0.1592 p=0.118	-0.0435 p=0.376	0.1852 p=0.088	0.0262 p=0.423	0.1382 p=0.153	-0.2099 p=0.060
% Energy - Carbohydrate	-0.0010 p=0.497	-0.1658 p=0.109	-0.1289 p=0.174	-0.1262 p=0.179	0.0212 p=0.438	-0.0335 p=0.402	0.1414 p=0.149
% Energy - Total Fat	-0.0194 p=0.443	0.1627 p=0.113	0.0251 p=0.428	0.1661 p=0.113	-0.0404 p=0.383	0.1166 p=0.194	0.356 p=0.397
% Energy - Saturated Fat	0.0175 p=0.449	0.3143 p=0.009	0.1517 p=0.134	0.2484 p=0.034	0.0889 p=0.255	0.2679 p=0.022	-0.2384 p=0.038
% Energy - Polyunsat. Fatty Acids	-0.0453 p=0.369	-0.1601 p=0.117	-0.1165 p=0.198	-0.1003 p=0.233	-0.1253 p=0.177	-0.0847 p=0.266	0.1915 p=0.079
% Energy - Linoleic Acid	-0.0212 p=0.438	-0.1649 p=0.110	-0.1127 p=0.206	-0.1146 p=0.202	-0.1175 p=0.192	-0.0777 p=0.283	0.1539 p=0.129
P:S Ratio	-0.0701 p=0.302	-0.2528 p=0.029	-0.1857 p=0.087	-0.1632 p=0.117	-0.1727 p=0.100	-0.2087 p=0.060	0.3050 p=0.011
Cholesterol	-0.072 p=0.479	0.2944 p=0.013	0.0624 p=0.325	0.2955 p=0.014	0.1794 p=0.091	0.1864 p=0.083	-0.1280 p=0.173
Starch	-0.1164 p=0.194	-0.2302 p=0.042	0.0954 p=0.244	-0.2805 p=0.019	-0.1088 p=0.210	-0.2368 p=0.038	-0.0917 p=0.251
Alcohol	0.0039 p=0.489	-0.0922 p=0.248	0.3250 p=0.008	-0.2299 p=0.046	-0.0178 p=0.448	-0.2757 p=0.019	-0.1887 p=0.082
Fibre	-0.0697 p=0.303	-0.2953 p=0.013	0.0735 p=0.297	-0.3325 p=0.007	-0.2193 p=0.051	-0.3597 p=0.003	-0.1249 p=0.180

TABLE 7:5

Multiple regression analysis for plasma triglyceride concentrations

Variables in Regression	Standard Regression Coefficient	T value	Significance of T value
<i>Biceps</i>	0.360	2.986	0.004
$R^2 = 0.129$			

Total Cholesterol

There was a significant positive correlation between total cholesterol concentrations in plasma and all anthropometric variables listed, with the exception of W/H ratio. However, the most significant correlation was found with age and subscapular skinfold thickness (Tables 7:3 & 7:4). Body fat also appears to have a significant relationship with plasma cholesterol levels. A strong positive correlation was found between dietary energy from saturated fat followed by dietary cholesterol. Significant negative correlations were also found with P:S ratio, starch and fibre intakes.

In the multivariate analysis, energy from saturated fat and subscapular and suprailiac thickness were identified as the major predictors of total cholesterol in plasma and 24.5% of the variation was explained by these variables (Table 7:6). But when subscapular thickness was excluded from the analysis, age was the second important variable in predicting cholesterol concentrations. There was a strong correlation (0.3137, $p=0.005$) between increasing age and subscapular thickness in these subjects. It is also well-known that cholesterol levels increase with age. The Caucasian vegetarians who were slightly younger in age had lower subscapular thickness and total cholesterol levels, which may explain the strong relationship found between subscapular skinfold and plasma cholesterol. However, it is also recognised that the Indian women had lower levels of cholesterol compared to omnivores, whose subscapular skinfolds were well below those of the Indian women. Several inter-country epidemiological studies have confirmed the influence of percentage of energy from saturated fatty acids and P:S ratio on plasma cholesterol, but very few studies have shown similar relationships within the population of a country. A significant relationship between saturated fat intake and total cholesterol, as well as with LDL-cholesterol and ApoB, was found in this study which was confirmed

by both univariate and multivariate analyses. It is of particular interest that the relationship between saturated fat intake and plasma cholesterol found in this study was similar to that predicted by Keys *et al.* (1965) in young men. However, no such relationship has been demonstrated in women so far.

TABLE 7:6

Multiple regression analysis for plasma cholesterol

Variables in Regression	Standard Regression Coefficient	T value	Significance of T value
<i>Energy from Saturated Fatty Acids</i>	0.281	2.275	0.027
<i>Subscapular Thickness</i>	0.646	3.113	0.003
<i>Suprailiac Thickness</i>	-0.442	-2.093	0.041
$R^2 = 0.245$			

HDL-Cholesterol

Individual nutrients did not appear to have a significant impact on HDL-cholesterol in this study. It is well-known that intake of alcohol is associated with an increase in HDL levels, and a positive correlation ($r=0.325$, $p=0.008$) was found between alcohol intake and HDL-cholesterol concentrations in these subjects. Studies on Indians have persistently reported lower HDL levels despite higher intakes of polyunsaturated fatty acids (McKeigue *et al.*, 1985; Miller *et al.*, 1988) and this has been confirmed in this study. There seems to be a strong negative relationship between body fat and HDL levels in these subjects. In addition to skinfold measurements, the W/H ratio was also negatively correlated with HDL-cholesterol concentrations (Tables 7:3 & 7:4). Similar results were reported by McKeigue *et al.* (1985) who found a stronger negative relationship between W/H ratio and HDL in men than in women.

In the multivariate analyses (Tables 7:7 & 7:8), percentage of body fat and intake of alcohol explained 41.1 % of the variance in total HDL levels in all subjects. W/H ratio was not a strong predictor of HDL-cholesterol concentrations in these women. However, multivariate analysis of HDL₂ revealed the percentage of body fat as the sole important predictor of HDL₂ levels. The results of this study show that although alcohol intake has

been known to increase HDL cholesterol, it may have little effect on the HDL₂ fraction which is believed to be protective against cardiovascular disease. This is in agreement with other studies (Frimpong and Lapp, 1989). The variation in HDL₂ concentrations of 31.8% could be explained by differences in body fat of these subjects, but not by the pattern of fat distribution.

TABLE 7:7

Multiple regression analysis for HDL-cholesterol concentrations

Variables in Regression	Standard Regression Coefficient	T value	Significance of T value
% Body Fat	-0.560	-5.192	0.000
Alcohol Intake	0.237	2.202	0.032
$R^2 = 0.411$			

TABLE 7:8

Multiple regression analysis for HDL₂ cholesterol

Variables in Regression	Standard Regression Coefficient	T value	Significance of T value
% Body Fat	-0.564	-4.877	0.000
$R^2 = 0.318$			

LDL-Cholesterol and ApoB

The dietary variables that showed significant correlation with total cholesterol in plasma appear to be related to LDL-cholesterol because 60-70% of total cholesterol in plasma is in the LDL fraction. Similarly, ApoB correlated significantly with the percentage of energy from saturated fat and showed a negative relationship between starch, alcohol and fibre intakes. Both LDL-cholesterol and ApoB significantly correlated with all measurements of body fat, with the exception of W/H ratio.

Subscapular skinfold measurement emerged as the most significant predictor of LDL-cholesterol in plasma, followed by dietary cholesterol (Table 7:9). The percentage

of energy from saturated fat, which was related to total plasma cholesterol, did not appear to have the same effect on LDL-cholesterol in this study. However, dietary cholesterol could predict 10% of the variation in LDL-cholesterol levels of these subjects. Dietary cholesterol is believed to down-regulate the LDL receptor activity (Després *et al.*, 1991). Fibre was the sole predictor of ApoB concentrations in this study, accounting for 13% of the variation in plasma (Table 7:10).

TABLE 7:9

Multiple regression analysis for plasma LDL-cholesterol

Variables in Regression	Standard Regression Coefficient	T value	Significance of T value
<i>Subscapular Thickness</i>	0.425	3.760	0.0004
<i>Dietary Cholesterol</i>	0.391	3.369	0.001
<i>Alcohol</i>	-0.266	-2.262	0.028
$R^2 = 0.368$			

TABLE 7:10

Multiple regression analysis for ApoB

Variables in Regression	Standard Regression Coefficient	T Value	Significance of T Value
<i>Dietary fibre</i>	-0.360	-2.859	0.006
$R^2 = 0.129$			

Apo(a)

Concentrations of Apo(a) are believed to be genetically determined and no studies in humans have shown any influence of dietary fat. However, in this study, univariate analysis revealed a negative correlation between Apo(a) levels and the percentage of energy from saturated fat, and a positive correlation with the P:S ratio of the diet.

Body fat and centripetal fat distribution at subscapular and suprailiac also correlated significantly with Apo(a) concentration. However, the W/H ratio did not have a significant relationship with Apo(a) concentrations but, in fact, was negatively correlated.

Multivariate analysis showed that both suprailiac skinfold and W/H ratio could explain 23% of the variation in Apo(a) levels of these subjects (Table 7:11). This shows that with increasing W/H ratio there may be a decrease in Lp(a) in plasma and thereby it could be assumed that a relationship exists between upper body obesity and Lp(a) concentrations in plasma, and this would explain the relationship between non-insulin dependent diabetes and elevated Lp(a) levels.

TABLE 7:11

Multiple regression analysis for plasma Apo(a) concentrations

Variables in Regression	Standard Regression Coefficient	T Value	Significance of T Value
<i>Suprailiac Thickness</i>	<i>0.460</i>	<i>3.815</i>	<i>0.0003</i>
<i>W/H Ratio</i>	<i>-0.312</i>	<i>-2.58</i>	<i>0.013</i>
<i>R² = 0.231</i>			

It has been argued that the relationship between most risk factor levels and CHD incidence is continuous and linear or curvilinear, without evidence of a threshold demarcating a boundary between low risk and high risk ranges (European Atherosclerosis Society, 1987). Therefore, an absolute cut-off point for desirable cholesterol may not be appropriate. According to the European Atherosclerosis Society, cholesterol levels of 5.2-6.5 mmol/l associated with normal triglyceride (<200 mg/dl) and total HDL (0.9 mmol/l) indicate a need for correction of risk factors, if present; particularly reduction of body weight and refraining from smoking. Forty-one percent of Caucasian omnivores, 18% of Indians and 17% of Caucasian vegetarians fell into this category (Table 7:12). Only 3 omnivores and one Indian vegetarian had total plasma cholesterol levels in the range of 6.5-7.8 mmol/l, levels at which use of lipid lowering drugs may need to be considered where dietary intervention is not successful. However, concentrations of triglycerides and total HDL-cholesterol were all within the acceptable ranges. Plasma

cholesterol levels were below 5.2 mmol/l in the majority of vegetarians and in approximately half the omnivores in this study. The results of this study show that Caucasian omnivores appear to be at a greater risk of CHD based on their cholesterol levels.

TABLE 7:12

Distribution of plasma cholesterol concentrations by group
Number of subjects with percentages in parentheses

	<5.2 mmol/l	5.2-6.5 mmol/l	6.5-7.8 mmol/l
<i>Caucasian Omnivores (n=22)</i>	10 (45.5)	9 (40.9)	3 (13.6)
<i>Indian Vegetarians (n=22)</i>	17 (77.3)	4 (18.2)	1 (4.5)
<i>Caucasian Vegetarians (n=18)</i>	15 (83.3)	3 (16.7)	0

7:4 Discussion

Most studies have also identified plasma concentrations of HDL as a powerful predictor and some also found LDL to be a good predictor of CHD risk. The omnivores in this study had higher plasma cholesterol, LDL and ApoB concentrations compared to both groups of vegetarians. There were marked differences in the lipid risk factors between the vegetarians. Indian vegetarians had intermediate levels of total cholesterol, LDL-cholesterol and ApoB associated with significantly lower levels of HDL. They appear to differ from the Caucasians by the presence of two important risk factors: low total HDL and HDL₂-cholesterol concentrations and high levels of Apo(a).

Apo(a) is a major constituent of Lp(a), the concentration of which has been recently recognised as a significant independent genetic risk factor for CHD. Rhoads *et al.* (1986) found men with elevated plasma Lp(a) were at increased risk of suffering a myocardial infarction by the age of 60. Durrington *et al.* (1988) argued that much of the genetic component of cardiac ischaemia, that is not expressed through any of the traditional risk factors, could be explained by Lp(a) concentrations. High levels of Lp(a) have also been associated with hyperinsulinism and NIDDMs. Seed *et al.* (1990) reported that elevated Lp(a) concentrations in patients with familial hypercholesterolaemia were associated with clinical CHD. There are few reports of Lp(a) as a potential risk

factor in women. Key and Sanders (personal communication), in a retrospective study in Guernsey, found a non-significant trend for Lp(a) concentration to be elevated in women who suffered myocardial infarction or angina. They found that plasma cholesterol, Apo A1 and BMI were significant predictors of risk. The higher concentrations found in the Indian women in this study would be consistent with an increased risk of CHD.

The HDL-cholesterol concentrations were significantly greater in the Caucasian vegetarians compared to the Indians, but not when compared to the omnivores. The HDL₂ fraction was significantly greater in Caucasian vegetarians when compared to both omnivores and Indians. Multivariate analysis showed a significant effect of alcohol on total HDL cholesterol concentrations but not that of HDL₂ cholesterol. The higher HDL₂ cholesterol in the Caucasian vegetarians cannot therefore be explained by the difference in alcohol intake.

Factors other than diet composition are known to influence HDL and its fractions in plasma, notably amount and distribution of body fat. Significant negative correlations between BMI, % Body Fat and all skinfold measurements and W/H ratio were found. This implies that there is a strong influence of body fat on total HDL and HDL₂ concentrations in these subjects which was also reinforced in the multiple regression model which included all anthropometric variables. Haines *et al.* (1987) reported that centrally located subcutaneous fat is more closely related to cardiovascular risk in women. Greater subscapular and suprailiac skinfold measurements in the Indians were associated with lower HDL levels in the present study. However, the waist/hip ratio did not differ between the groups which may mean that total body fat and obesity influenced HDL levels regardless of waist/hip ratio. According to Depres *et al.* (1990) intra-abdominal fat deposition is associated with reduced HDL levels in obese women but not in the non-obese and they concluded that at least in pre-menopausal women obesity has to be present for there to be a significant association between abdominal fat and plasma lipoprotein concentrations. However, in the present study a significant negative correlation was found between the waist/hip ratio and HDL concentrations. The majority of the subjects were non-obese, yet strong correlations were also found between the proportion of body fat and all the plasma lipoproteins with the exception of Apo A1.

The LDL-cholesterol fraction forms 50-60% of the total cholesterol in plasma and as ApoB is related to LDL-cholesterol it could be argued that similar dietary factors are implicated in determining their plasma concentrations. The results of the present study show that LDL-cholesterol is related to dietary cholesterol and alcohol intake, whereas fibre intakes could solely explain 13% of the variation in ApoB levels. This suggests that dietary factors may influence ApoB secretion independently.

In addition to dietary factors influencing cholesterol and LDL cholesterol, body fat appears to be significantly related to their levels in plasma. The significant correlations between body fat and subcutaneous fat in women observed in this study agree with the observations made by Haines *et al.* (1987), who also found a significant effect of skinfold thickness on cholesterol and other cardiovascular risk factors. However, Depres *et al.* (1990) observed that in non-obese women, a high trunk abdominal fat accumulation measured by computer topography was associated with increased LDL cholesterol and ApoB levels. They concluded that in non-obese, pre-menopausal women, subcutaneous trunk-abdominal fat accumulation and abdominal cell hypertrophy are related to changes in plasma lipoprotein levels, whereas the W/H ratio and the deep abdominal fat show little association with the metabolic profile of lean women. In this study, the W/H ratio was also not a predictor of cholesterol, LDL-cholesterol or ApoB levels, but subcutaneous fat measurements were.

Indian vegetarians, despite their lower intake of saturated fat and higher P:S ratio in the diet, showed a higher prevalence of lipoprotein risk factors for CHD. Centripetal fat distribution associated with lower HDL₂ and higher triglycerides, LDL and ApoB concentrations observed in Indians in this study are consistent with other studies on this group. The dietary intake of tocopherol and its concentrations in plasma were significantly lower in the Indians. This observation, in association with higher levels of LDL compared to Caucasian vegetarians, may be of importance because there seems to be an inverse correlation between absolute levels of α -tocopherol and mortality from CHD (Gey *et al.*, 1991). Also, since the differences in lipid profiles persisted between the vegetarian groups, it may be that other factors such as body fat and its distribution pattern overtake the influence of diet composition in the Indians; whereas in the Caucasians diet may be an influential factor in determining lipoprotein concentrations in plasma. Although the Indian vegetarians could not be classed as being obese, their

android distribution of fat and greater percentage of body fat may indicate a tendency towards obesity which could be the underlying factor in disturbances of lipoprotein metabolism.

7:5 Summary

1. Retinol and α -tocopherol concentrations in plasma were measured. The Indian vegetarians had significantly lower levels of retinol compared to the Caucasians. α -Tocopherol concentrations were similar in Caucasian omnivores and the Indians and significantly lower than in the Caucasian vegetarians.
2. Plasma concentrations of cholesterol, LDL-cholesterol and ApoA^I were significantly greater in the omnivores compared to the Caucasian vegetarians. The Indian vegetarians had significantly lower HDL and HDL₂ cholesterol and greater levels of Apo(a) compared to both groups of Caucasians. Concentrations of ApoB were significantly lower and those of HDL₂-cholesterol greater in the Caucasian vegetarians compared to both the omnivores and Indians.
3. Univariate and multivariate analyses revealed that body fat and its distribution, especially upper body skinfold measurements, were significantly related to lipoprotein concentrations in plasma.
4. Among dietary variables the energy from saturated fatty acids was related to cholesterol levels in plasma, dietary fibre was significantly related to ApoB concentrations, and dietary cholesterol and alcohol intakes were related to LDL-cholesterol.
5. It is concluded that in the Indians body fat and its pattern of distribution considerably influence lipoprotein concentrations rather than the diet composition.

CHAPTER 8

SEX HORMONE CONCENTRATIONS IN PLASMA

8:1 Introduction

Sex hormones are believed to be influenced by diet, both directly in terms of dietary quality and indirectly by the proportion of fat in the body. They play an essential role in the expression of secondary sexual characteristics. However, variations in sex hormone levels are related to disease. Oestrogens are believed to exert a protective effect against osteoporosis, CHD and ovarian cancer, but may increase risk of endometrial and breast cancer. Lower rates of osteoporosis (Marsh *et al.*, 1988) have been reported in Caucasian vegetarians compared with omnivores. This has been attributed to an effect of boron present in fruit and vegetables increasing oestrogenic activity. Nielsen *et al.* (1987) reported that boron supplementation markedly elevated serum concentrations of oestradiol and testosterone. The influence of vegetarian diets on CHD has previously been discussed in Chapter 1. Ovarian and breast cancer were similar in vegetarian and non-vegetarian Seventh-Day Adventists (Snowden, 1988). Kinlen *et al.* (1983) reported a higher than expected proportional mortality rate of breast cancer in British Caucasian vegetarian women, but this was consistent with the high proportion of cancer in women. Mortality rates from breast cancer are lower in Indian women than in the general population. This may be due to differences in reproductive history (i.e. early age of first pregnancy, late menarche) or dietary and genetic factors.

In this chapter plasma oestrogen, testosterone and SHBG concentrations are compared between Indian vegetarians, Caucasian vegetarians and omnivores and the inter-relationships with plasma lipids, anthropometric and dietary variables are discussed.

8:1:1 Synthesis and Metabolism of Sex Hormones

Steroid hormones in females are synthesized either in the ovary or in the adrenal gland, but many other tissues contribute to their metabolism. The synthesis starts from cholesterol where the side-chain at C17 is cleaved to give Δ -5-pregnenalone which can follow two pathways (Figure 8:1), either via progesterone (Pg) or via dehydroandrosterone to form testosterone and oestradiol.

The metabolism of oestradiol (Figure 8:2) is almost exclusively oxidative in nature, involving a series of hydroxylations and oxidations. Oestradiol (E2), the primary ovarian hormone secretory product, is first oxidised to oestrone (E1) followed by a series of reversible and irreversible reactions. Oestrone is the linchpin of this combination of

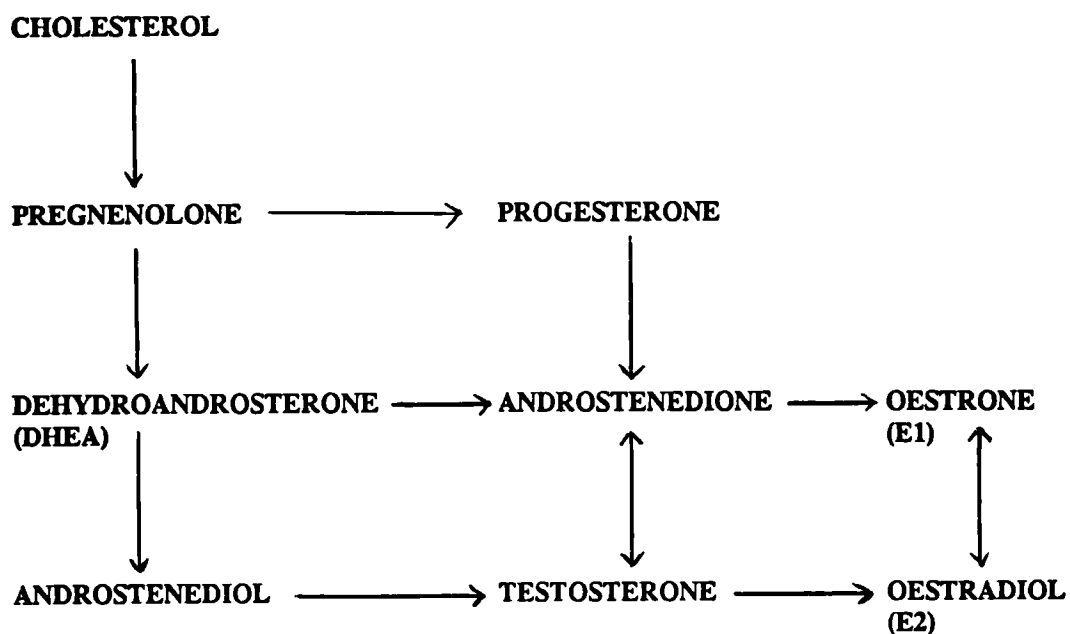


Figure 8:1

Biosynthesis of sex steroid hormones

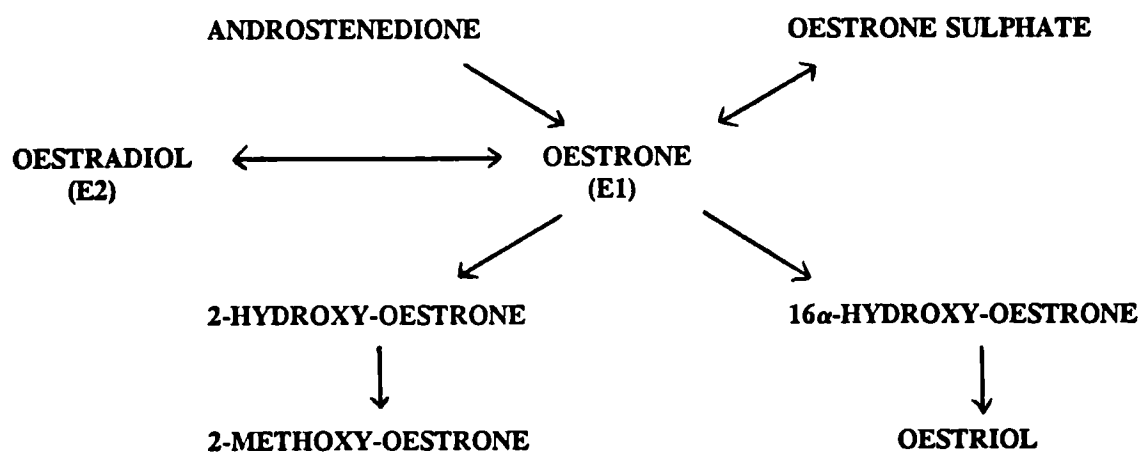


Figure 8:2

Pathways of oestrogen formation and metabolism

reactions and the principal precursor for the irreversible reactions. The 16α -pathways leads to oestriol via 16α -hydroxyoestrone; the alternative hydroxylation at C-2 yields the catechol oestrogens and these two reactions are essentially mutually exclusive and obtain physiologic significance in that the products of these pathways have distinctly different biological properties (Fishman and Martucci, 1980).

The 2-hydroxy-oestrogens, primarily 2-hydroxyoestrone and 2-methoxy-oestrogen, are virtually devoid of peripheral oestrogenic activity as measured by bio-assay. In contrast, oestrone and its 16α -hydroxylated products display potent peripheral oestrogenic activity. The 16α -hydroxylated products also have minimal affinity for sex hormone binding globulin (SHBG), which serves to potentiate the impact of these compounds on oestrogen target tissues in humans (Fishman and Martucci, 1980). The principal oestrogenic hormone in circulation is oestradiol and the most active sex hormones are bound entirely to plasma proteins to prevent diffusion into tissues and rapid clearance. About 66% of testosterone (T) and 36% of oestradiol is bound to SHBG, whereas 30% and 60% respectively are bound to albumin and 1% and 2% respectively are transported free (Berrino *et al.*, 1988).

The ovary produces oestrogens, androgens and progesterone. The interstitial cells are probably a source of oestrogens but developing follicles are the major source. Some testosterone is produced, but more is formed by peripheral conversion. Progesterone is secreted mainly by the *Corpus luteum*. The principal oestrogen secreted by the ovary is oestradiol but oestrone may be present in the plasma in a similar concentration. The secretion of ovarian hormones is under the control of the anterior pituitary hormones: luteinizing hormone (LH) and follicle stimulating hormone (FSH). The metabolism of oestradiol is principally by the liver to a relatively inactive compound oestriol, which is conjugated with glucouronic acid and excreted in urine. Progesterone is converted to pregnanediol and excreted in urine, also as a glucouronide.

The control of the normal menstrual cycle depends on a complex pattern of interactions of the ovarian hormones. During menstruation the ovarian follicles mature, leading to a follicular phase when oestrogen production rises and also the plasma level. The endometrium proliferates and the cervical mucus increases in amount and viscosity. The rising plasma oestrogen reaches a critical level, above which there is a sudden surge

of LH accompanied by a smaller rise in FSH. The LH surge causes rupture of the follicle and ovulation occurs and the developing *Corpus luteum* secretes increasing amounts of progesterone. During the luteal phase the levels of both oestrogens and progesterone are high, with progesterone opposing the action of oestrogens. The luteal phase ends with a fall in the progesterone levels leading to the onset of menstruation.

8:1:2 Hormonal Theories Concerning Breast Cancer

Many observations implicate sex hormones as having a role in cancer of reproductive tissues, including the breast, endometrium, cervix and ovary. They may be involved in the initiation as well as the promotion of tumours. At least 12 hormonal hypotheses for breast cancer have been proposed (Berrino *et al.*, 1988). The strong protective effect of early menopause or oophorectomy, as well as the increased risk associated with early menarche, argue for a major role of one or both of the principal ovarian hormones (progesterone and oestrogen). The risk of developing breast cancer before the age of 40 is lower in nulliparous than in parous women, but above this age the relationship is reversed so that parous women have a substantially lower lifetime risk than nulliparous women (Janesich and Hoff, 1982). Early age at first birth is associated with decreased risk at these older ages and increasing parity appears to cause further small decreases in risk.

It has been argued that progesterone alone may afford some protection by opposing the action of oestrogens. Recently it was reported (Badwe *et al.*, 1991) that timing of surgery in pre-menopausal breast cancer patients was important. Surgery undertaken in women during the follicular phase of unopposed oestrogen stimulation had reduced survival and actuarial survival at ten years was only 54% compared to 84% in those who underwent surgery during the luteal phase, when oestrogen activity is opposed by progesterone. Key and Pike (1988) suggest that the hormonal pattern of pre-menopausal women (cyclical production of relatively large amounts of E2 and Pg) causes a greater rate of increase in risk of breast cancer than the hormonal pattern in post-menopausal women (constant low E2 and very low Pg). Breast cancer cases have been shown to have shorter menstrual cycles than controls (Olsson *et al.*, 1983; La Vecchia *et al.*, 1985), therefore the total number of cycles and the frequency of exposure to high oestrogen and progesterone levels throughout the reproductive phase may be an important factor in determining breast cancer risk.

The effects of oestrogens and progestogens on breast cell division in cell culture systems were studied by McManus and Welsch (1984) who found that oestradiol alone caused a large increase in DNA synthesis, while progestogen alone caused a small increase, and both together have a similar effect to that of oestradiol alone. Key and Pike (1988) suggest a possible "oestrogen and progestogen hypothesis". They argue that breast cell proliferation is increased by the combined effects of oestrogen and progesterone and that the duration of the luteal phase is a critical factor in determining risk of breast cancer. It is argued that increased mitogenesis increases the risk of mutagenesis. This hypothesis is supported by the following observations: post-menopausal women receiving oestrogen and progestogen hormone replacement therapy have a greater risk of breast cancer than those receiving oestrogen alone; oral contraceptives providing both oestrogen and progestogen fail to protect against breast cancer.

Adrenal or gonadal androgens can be converted to oestrone and subsequently to oestradiol in peripheral tissue, particularly adipose tissue. The peripheral conversion of androgens to oestrogens plays an important part in the normal expression of secondary sexual characteristics. Frisch (1984) showed that a certain critical level of body fat (15-18%) was necessary to support normal menstruation and suppress facial hair from developing in females. Obesity, on the other hand, can lead to excessive conversion of androgens to oestrogens and lead to suppression of menstruation. In post-menopausal women extra-gonadal oestrogen synthesis is predominant. The relationship between obesity and breast cancer in post-menopausal women may be partly explained by high oestrogen production.

Sex hormones mainly express biological activity in the unbound or free form. Moore *et al.* (1982) showed that breast cancer cases had higher levels of free E2 compared with controls and that the daughters of cases also showed higher levels of E2. Oestradiol binds to SHBG with a lower affinity than the androgens, particularly testosterone. It is thought that high testosterone levels may decrease SHBG production and because of its greater affinity for SHBG it may displace E2 from the protein, thereby increasing its free fraction in plasma. Plasma concentrations of SHBG are also influenced by obesity. Low levels of SHBG are common in subjects with upper body obesity and lean subjects tend to have higher plasma concentrations of SHBG. However,

homeostatic mechanisms exist which compensate for variations in plasma SHBG (Key *et al.*, 1990) so that the plasma concentration of free sex hormones is kept relatively constant.

It has also been shown that many post-menopausal women with breast cancer have tumours with receptors to oestrogen. In contrast, in pre-menopausal women, the majority of tumours are oestrogen receptor negative. This would be consistent with the observation that tamoxifen, a drug that antagonises the action of oestrogen, inhibits tumour growth in post-menopausal women (Early Breast Cancer Trialist's Collaborative Group, 1988). Another explanation why Tamoxifen probably is without benefit in pre-menopausal women may be because its effects are overwhelmed by the abundance of ovarian oestrogen.

8:1:3 Influence of Diet on Sex Hormones

Dietary influences of fat, fibre and vegetarianism on sex hormones have been studied by several workers. Reduction of dietary fat was shown to increase the length of the menstrual cycle (Jones *et al.*, 1987), but precise ovulation dates and duration of follicular and luteal phases were not determined. Details of some intervention studies on the effects of dietary fat reduction on sex hormone levels are given in Table 8:1. All the studies were small and lacked the necessary statistical power to detect small changes in hormone levels, which might well be biologically significant. A majority of these were done on student volunteers or on women with cyclical mastopathy and the results are difficult to interpret.

Longcope *et al.* (1987) reported a redistribution of urinary metabolites of oestrogen from the 16α -hydroxyoestrone pathway towards the catechol-oestrogen pathway in subjects on a low fat diet. This might imply that fat intake may affect the balance of oestrogen metabolites. Woods *et al.* (1989) noted lower concentrations of oestrone sulphate in women on a low fat diet.

Several studies on vegetarians and non-vegetarians have reported differences in sex hormone levels in plasma and their excretion in urine and faeces. Pre-menopausal Seventh-Day Adventists lacto-vegetarians ($n=14$) had lower plasma levels of oestrone and oestradiol during the mid-luteal phase of the menstrual cycle compared to non-vegetarians

TABLE 8:1 Details of some intervention studies on the effect of dietary fat reduction on sex hormone levels

	Ingram <i>et al.</i> (1987)			Rose <i>et al.</i> (1987)			Boyd <i>et al.</i> (1988)			Woods <i>et al.</i> (1989)			Williams <i>et al.</i> (1989)		
	Control	Inter- vention	P	Control	Inter- vention	P	Control	Inter- vention	P	Control	Inter- vention	P	Control	Inter- vention	P
<i>N</i>	9	9		16	same		10	11		17	same		15	same	
<i>Time on diet</i> (wks)	8	8		4	12		24	24		4	8-10		8	8	
% fat	40	18		35	21		33	23		40	25		37	21	
<i>Energy (MJ)</i>	7.2	5.6		7.3	5.6		7.0	6.3		iso- caloric	iso- caloric		7.8	6.1	
<i>P : S</i>	-	-		0.52	0.56		0.66	0.61		0.5	1.0		0.38	0.61	
<i>Fibre (g/d)</i>	19	21		14	6		-	-		12	40		22	28	
<i>Weight (kg)</i>	62.0	62.2		59.4	58.0		58.1	59.6		60.5	same		64.2	61.1	
<i>Day of cycle</i>	21	21		17-20	same		luteal	luteal		4-7	same		21-26	same	
<i>E1 (pmol/l)</i>	-	-		611	363	<.005	296	301	NS	188	200	NS	-	-	<.05
<i>E2 (pmol/l)</i>	423	467	0.70	492	371	<.01	330	353	NS	223	221	NS	440	385	
<i>T (nmol/l)</i>	1.5	1.1	0.11	-	-		-	-		1.4	1.1	NS	-	-	
<i>SHBG (nmol/l)</i>	44	52	0.20	-	-		0.96*	0.95	NS	80	61	NS	-	-	
<i>E1 sulphate</i> (nmol/l)	-	-		-	-		-	-		2.1	1.3	<.001	-	-	

* Units not given

(n=9) (Schultz and Leklem, 1983). However, the sample size of the study was small and neither the biologically active free oestradiol nor SHBG were measured.

Gray *et al.* (1982) found no differences in plasma or urinary levels of sex hormones in Seventh-Day Adventist 'vegetarian' and 'non-vegetarian' girls aged 14-17. Pre-menopausal vegetarian women were reported to have lower concentrations of oestrone and oestradiol and slightly higher concentrations of testosterone and androstenedione compared to the non-vegetarians, but none of these differences achieved statistical significance (Goldin *et al.*, 1982). However, there was an inverse relationship between the plasma oestrogen level and faecal excretion of oestrogens which was statistically significant. The interpretation of this study is difficult, particularly as the authors also report 7 g/day intake of animal protein from fish in the vegetarian group. Goldin *et al.* (1986) found that Oriental omnivorous pre-menopausal and post-menopausal women who ate low fat and low meat diets had lower plasma oestrogen concentrations than did age-matched Caucasian omnivorous women. They also had a higher excretion of oestrogens compared to the Caucasians.

Post-menopausal vegetarians have been reported to have lower urinary oestriol and total oestrogens, low plasma prolactin, higher SHBG and HDL-cholesterol, even after adjustment for weight, age and alcohol consumption (Armstrong *et al.*, 1981). Barbosa *et al.* (1990) found significantly lower levels of oestradiol and SHBG in elderly Seventh-Day Adventist vegetarian women compared to non-vegetarians from the same sect.

Several studies have examined SHBG concentrations in vegetarian men. Hämäläinen *et al.* (1984) observed a non-significant 9% decrease in SHBG in men changing to a semi-vegetarian diet. Belanger *et al.* (1989) found that mean SHBG was 47% higher in the vegetarians, but it is difficult to interpret this result because the mean BMI of the vegetarians was 11% lower than that of the omnivores and BMI is strongly inversely related to SHBG (Moore and Bulbrook, 1988). Key *et al.* (1990) reported 23% higher SHBG in vegans than in omnivores after adjusting for BMI and suggest that the dietary intake of phyto-oestrogens could be the cause of high SHBG levels, since a low correlation was found between SHBG and dietary fibre. However, a positive correlation between SHBG and fibre was found in both men (Bishop *et al.*, 1988) and women (Adlercreutz *et al.*, 1987).

8:2 Methods

The protocol and analytical methods are described in detail in Chapter 2. Significance of differences in sex hormone levels were tested by one-way analysis of variance and Duncan's multiple range test. Relationships between variables were tested by univariate and multivariate analyses.

8:3 Results

The characteristics of subjects related to their reproductive history are given in Table 8:2.

TABLE 8:2

Reproductive history and oral contraceptive use in the subjects

	CO		IV		CV	
<u>Age of Menarche</u> (no. of subjects (%))						
10+ to 13 years	14	(58.3)	11	(45.8)	8	(44.4)
13+ to 15 years	8	(33.3)	13	(54.2)	9	(50.0)
Over 15 years	0		0		1	(5.6)
<u>Mean (SEM) age at first childbirth</u>	25.5 (n=19)	(1.16)	23.0 (n=20)	(1.02)	29 (n=1)	
<u>Mean (SEM interval (yrs) since recent childbirth</u>	6.3	(1.08)	8.5	(1.15)	2.0	
<u>Use of Oral Contraceptives</u> no. of subjects (%) who are:						
Current users	3	(12.5)	1	(4.2)	4	(22.2)
Past users	18	(75.0)	13	(54.2)	11	(45.8)

A majority of the Caucasian omnivorous women appeared to have attained menarche at a younger age compared to both groups of vegetarians. All the Caucasian vegetarians were nulliparous with the exception of one subject whose first childbirth was also at a later age compared to the other groups. Mean age of first childbirth was the lowest in the Indians, but was not significantly different from the omnivores. The mean interval of time since the most recent childbirth was also longer in the Indians compared to Caucasians. The use of oral contraceptives was more popular with Caucasians. Indians who had used oral contraceptives in the past had first started later in life (i.e. in their twenties) compared to Caucasians and the total duration of use was also shorter.

Table 8:3 gives the mean concentrations of sex hormones and SHBG in the Caucasian omnivores, Indian and Caucasian vegetarians. Mean values were also adjusted for age, BMI and oral contraceptive use. Total oestradiol and free E2 levels were higher in the omnivores but the differences were not statistically significant. Oestradiol concentrations, both total and free, were almost similar in both groups of Caucasians after adjustment for age, BMI and oral contraceptives, while the concentrations in the Indians were lower. Caucasian vegetarians differed from the omnivores and Indians in their significantly greater levels of total and free testosterone, which remained high even after adjusting the co-variables. Both groups of Caucasians had greater levels of SHBG compared to the Indians, but only the differences between omnivores and Indians achieved statistical significance. When both sets of Caucasians were grouped together, the SHBG levels in the Indians were significantly lower. After adjusting for the co-variables, SHBG levels were quite similar in both groups of Caucasians. The free oestradiol/testosterone ratio was significantly greater in the Indians despite lower concentrations of oestradiol and testosterone in this group. These differences in ratios between groups remained even after controlling for age, BMI and oral contraceptive use.

Univariate analysis was undertaken to examine the relationship between sex hormone concentrations and other variables. Tables 8:4 to 8:7 give Pearson's correlation coefficient between variables and also their probability values. Significant correlations ($p > 0.05$) observed for hormones and SHBG were included in their respective multiple regression models to identify the main predictors of hormones in these subjects.

TABLE 8:3

Sex hormone levels in plasma

	CO (n=22)		IV (n=21)		CV (n=18)	
	Mean	SEM	Mean	SEM	Mean	SEM
<i>Oestradiol (nmol/l)</i>	0.71	0.119	0.61	0.096	0.59	0.12
<i>Adjusted*</i>	0.68		0.56		0.69	
<i>Free oestradiol (pmol/l)</i>	11.9	2.0	11.5	1.90	10.2	2.2
<i>Adjusted*</i>	11.4		10.7		11.8	
<i>Testosterone (nmol/l)</i>	1.32 ^a	0.115	1.04 ^a	0.122	1.89 ^b	0.161
<i>Adjusted*</i>	1.33		1.01		1.91	
<i>Free testosterone (pmol/l)</i>	27.5 ^a	3.15	24.5 ^a	3.12	38.7 ^b	4.18
<i>Adjusted*</i>	28.3		23.7		38.6	
<i>SHBG (nmol/l)</i>	57.9 ^a	5.38	41.7 ^{b+}	5.12	55.8	4.76
<i>Adjusted*</i>	56.5		42.3		56.7	
<i>Free E2/Free T ratio</i>	0.48	0.070	0.61 ^a	0.131	0.26 ^b	0.055
<i>Adjusted*</i>	0.45		0.58		0.34	

^{a,b} Values with unlike superscript letters are significantly different from each other

⁺ Significantly different from all Caucasians

^{*} Adjusted for oral contraceptive use, age and BMI

TABLE 8:4 Correlation of sex hormones with anthropometric variables (r, p value)

Anthropometric variables	Total Oestrogen	Free Oestrogen	Total Testosterone	Free Testosterone	SHBG
<i>Height</i>	0.0799 <i>p</i> =0.270	0.1079 <i>p</i> =0.204	0.2295 <i>p</i> =0.038	0.2502 <i>p</i> =0.026	-0.1321 <i>p</i> =0.155
<i>Weight</i>	0.0383 <i>p</i> =0.385	0.0932 <i>p</i> =0.237	0.1478 <i>p</i> =0.128	0.2939 <i>p</i> =0.011	-0.3491 <i>p</i> =0.003
<i>BMI</i>	0.0139 <i>p</i> =0.458	0.0669 <i>p</i> =0.304	0.0318 <i>p</i> =0.404	0.1970 <i>p</i> =0.064	-0.3482 <i>p</i> =0.003
<i>Body fat</i>	-0.0804 <i>p</i> =0.269	-0.408 <i>p</i> =0.377	-0.2805 <i>p</i> =0.014	-0.1387 <i>p</i> =0.143	-0.2587 <i>p</i> =0.022
<i>Biceps Skinfold</i>	-0.0556 <i>p</i> =0.335	-0.0479 <i>p</i> =0.357	-0.0976 <i>p</i> =0.227	-0.0192 <i>p</i> =0.442	-0.1189 <i>p</i> =0.181
<i>Triceps Skinfold</i>	-0.0765 <i>p</i> =0.279	-0.0575 <i>p</i> =0.330	-0.3097 <i>p</i> =0.008	-0.2137 <i>p</i> =0.049	-0.0500 <i>p</i> =0.351
<i>Subscapular Thickness</i>	-0.0233 <i>p</i> =0.429	0.0177 <i>p</i> =0.446	-0.2410 <i>p</i> =0.031	-0.1076 <i>p</i> =0.205	-0.2954 <i>p</i> =0.010
<i>Suprailiac Thickness</i>	-0.0388 <i>p</i> =0.383	0.0203 <i>p</i> =0.438	-0.1329 <i>p</i> =0.154	0.0266 <i>p</i> =0.419	-0.3960 <i>p</i> =0.001
<i>Sum of skinfolds</i>	-0.0531 <i>p</i> =0.342	-0.0086 <i>p</i> =0.474	-0.2288 <i>p</i> =0.038	-0.0808 <i>p</i> =0.268	-0.2946 <i>p</i> =0.011
<i>Waist/hip ratio</i>	0.2147 <i>p</i> =0.048	0.2650 <i>p</i> =0.020	0.1357 <i>p</i> =0.149	0.2526 <i>p</i> =0.025	-0.3106 <i>p</i> =0.007

TABLE 8:5 Correlation of sex hormones with plasma lipoproteins (r, p value)

Plasma lipoproteins	Total Oestrogen	Free Oestrogen	Total Testosterone	Free Testosterone	SHBG
<i>Triglycerides</i>	-0.1711 <i>p</i> =0.094	-0.1218 <i>p</i> =0.175	-0.2217 <i>p</i> =0.039	-0.1147 <i>p</i> =0.189	-0.1461 <i>p</i> =0.131
<i>Total cholesterol</i>	0.0949 <i>p</i> =0.233	0.0608 <i>p</i> =0.321	-0.0845 <i>p</i> =0.259	-0.1040 <i>p</i> =0.213	0.0229 <i>p</i> =0.431
<i>HDL cholesterol</i>	0.0638 <i>p</i> =0.317	0.0259 <i>p</i> =0.424	0.1234 <i>p</i> =0.178	-0.0003 <i>p</i> =0.499	0.2851 <i>p</i> =0.015
<i>HDL₂ cholesterol</i>	0.2154 <i>p</i> =0.055	0.1781 <i>p</i> =0.095	0.2204 <i>p</i> =0.051	0.1315 <i>p</i> =0.167	0.1282 <i>p</i> =0.173
<i>HDL₃ cholesterol</i>	-0.1109 <i>p</i> =0.208	-0.1327 <i>p</i> =0.165	-0.0520 <i>p</i> =0.352	-0.1486 <i>p</i> =0.137	0.2645 <i>p</i> =0.024
<i>LDL cholesterol</i>	0.1380 <i>p</i> =0.151	0.1042 <i>p</i> =0.218	-0.0912 <i>p</i> =0.248	-0.0996 <i>p</i> =0.229	-0.0325 <i>p</i> =0.404
<i>Apo AI</i>	-0.0279 <i>p</i> =0.416	-0.0431 <i>p</i> =0.371	-0.2909 <i>p</i> =0.011	-0.2898 <i>p</i> =0.012	0.0759 <i>p</i> =0.281
<i>Apo B</i>	0.1066 <i>p</i> =0.207	0.0927 <i>p</i> =0.239	-0.2473 <i>p</i> =0.027	-0.2016 <i>p</i> =0.060	0.0866 <i>p</i> =0.253
<i>Apo(a)</i>	-0.0215 <i>p</i> =0.436	-0.006 <i>p</i> =0.498	-0.0366 <i>p</i> =0.391	0.0035 <i>p</i> =0.490	-0.1456 <i>p</i> =0.136

TABLE 8:6 Correlations of sex hormones with nutrient intakes (r, p value)

Nutrients	Total Oestrogen	Free Oestrogen	Total Testosterone	Free Testosterone	SHBG
Energy	-0.0524 p=0.351	-0.0734 p=0.295	0.3451 p=0.005	0.3024 p=0.012	0.0342 p=0.401
Fat	-0.2008 p=0.069	-0.2295 p=0.044	0.2114 p=0.059	0.1682 p=0.108	0.0507 p=0.355
% Energy from fat	-0.2966 p=0.013	-0.3228 p=0.008	-0.0853 p=0.266	-0.1177 p=0.194	0.0812 p=0.276
Saturated fat	-0.0886 p=0.258	-0.1519 p=0.132	0.0658 p=0.315	-0.0063 p=0.481	0.1874 p=0.083
% Energy from saturated fat	-0.0878 p=0.260	-0.1591 p=0.121	-0.0932 p=0.247	-0.1699 p=0.105	0.2371 p=0.039
Dietary cholesterol	0.0717 p=0.300	0.0306 p=0.411	0.2731 p=0.021	0.2159 p=0.055	0.1156 p=0.198
Protein	0.0970 p=0.239	0.0782 p=0.283	0.2130 p=0.058	0.1931 p=0.077	0.0375 p=0.392
% Energy from protein	0.1943 p=0.076	0.1847 p=0.086	-0.0151 p=0.456	-0.0026 p=0.492	0.0416 p=0.380
Carbohydrate	0.0974 p=0.238	0.0912 p=0.252	0.3126 p=0.010	0.3008 p=0.012	-0.0491 p=0.360
% Energy from carbohydrate	0.2505 p=0.031	0.2705 p=0.022	0.0426 p=0.378	0.0878 p=0.260	-0.1603 p=0.119
Sugars	0.2585 p=0.027	0.2403 p=0.037	0.2338 p=0.041	0.2214 p=0.051	-0.0497 p=0.358
Starch	-0.0508 p=0.355	-0.0625 p=0.324	0.3730 p=0.002	0.3144 p=0.009	0.0604 p=0.329
Fibre	-0.0111 p=0.468	0.0166 p=0.452	0.4814 p=0.000	0.4741 p=0.000	-0.0850 p=0.267
Alcohol	-0.2159 p=0.055	-0.2149 p=0.056	0.1186 p=0.192	0.0505 p=0.356	0.1854 p=0.086

TABLE 8:7 Correlation of sex hormones with foods consumed (r, p value)

Food groups	Total Oestrogen	Free Oestrogen	Total Testosterone	Free Testosterone	SHBG
<i>Cereals</i>	-0.0566 <i>p</i> =0.339	-0.0802 <i>p</i> =0.279	0.0851 <i>p</i> =0.266	0.0300 <i>p</i> =0.413	0.1567 <i>p</i> =0.124
<i>Milk and milk products</i>	0.0636 <i>p</i> =0.321	0.0631 <i>p</i> =0.322	-0.0259 <i>p</i> =0.425	-0.0348 <i>p</i> =0.399	-0.0921 <i>p</i> =0.250
<i>Eggs and egg products</i>	-0.2604 <i>p</i> =0.026	-0.2589 <i>p</i> =0.027	0.0159 <i>p</i> =0.454	0.0508 <i>p</i> =0.355	-0.0516 <i>p</i> =0.353
<i>Fat spreads</i>	-0.1757 <i>p</i> =0.098	-0.2078 <i>p</i> =0.062	0.2297 <i>p</i> =0.044	0.1919 <i>p</i> =0.078	0.0945 <i>p</i> =0.244
<i>Meat</i>	0.0400 <i>p</i> =0.385	0.0137 <i>p</i> =0.460	-0.0548 <i>p</i> =0.344	-0.0579 <i>p</i> =0.336	0.0966 <i>p</i> =0.239
<i>Meat and offal products</i>	0.3536 <i>p</i> =0.004	0.3203 <i>p</i> =0.008	0.0419 <i>p</i> =0.380	0.0454 <i>p</i> =0.370	0.0429 <i>p</i> =0.377
<i>Fish and fish products</i>	0.2123 <i>p</i> =0.058	0.2155 <i>p</i> =0.055	0.0925 <i>p</i> =0.249	0.1019 <i>p</i> =0.228	-0.0333 <i>p</i> =0.404
<i>Vegetables</i>	0.0848 <i>p</i> =0.267	0.0746 <i>p</i> =0.292	0.2512 <i>p</i> =0.031	0.2555 <i>p</i> =0.029	-0.0700 <i>p</i> =0.304
<i>Pulses</i>	-0.1883 <i>p</i> =0.082	-0.1543 <i>p</i> =0.128	0.0994 <i>p</i> =0.233	0.1346 <i>p</i> =0.161	-0.1240 <i>p</i> =0.181
<i>Fruit</i>	0.2164 <i>p</i> =0.055	0.2507 <i>p</i> =0.031	0.2503 <i>p</i> =0.031	0.2938 <i>p</i> =0.014	-0.1500 <i>p</i> =0.135
<i>Nuts</i>	-0.0408 <i>p</i> =0.383	-0.0233 <i>p</i> =0.432	-0.0142 <i>p</i> =0.459	-0.0074 <i>p</i> =0.478	-0.0051 <i>p</i> =0.485
<i>Sugars and preserves</i>	0.2616 <i>p</i> =0.026	0.2065 <i>p</i> =0.063	-0.0080 <i>p</i> =0.477	-0.0324 <i>p</i> =0.406	0.0364 <i>p</i> =0.395
<i>Non-alcoholic drinks</i>	0.0963 <i>p</i> =0.240	0.0667 <i>p</i> =0.313	0.1807 <i>p</i> =0.091	0.1336 <i>p</i> =0.163	0.1422 <i>p</i> =0.148
<i>Miscellaneous foods</i>	-0.0382 <i>p</i> =0.390	-0.0066 <i>p</i> =0.481	-0.0603 <i>p</i> =0.329	-0.0370 <i>p</i> =0.393	-0.1876 <i>p</i> =0.083

In the multiple regression analysis for both oestradiol and free oestradiol, diet appeared to have a significant influence (Tables 8.8 & 8.9). Consumption of meat and offal products, eggs and the percentage of energy from carbohydrate were the significant predictors of total oestradiol levels in these subjects, and 37.6% of the variation could be explained by the above variables. The waist/hip ratio, which was significantly correlated with both oestradiol and free oestradiol, was not a significant predictor of total oestradiol levels. However, the waist/hip ratio was the only significant anthropometric variable found in the regression model for free oestradiol, along with other dietary variables.

TABLE 8:8

Multiple regression analysis for oestradiol

Variables in Regression	Standard Regression Coefficient	T value	Significance of T value
<i>Meat and offal products</i>	0.566	4.726	0.000
<i>% Energy from carbohydrate</i>	0.413	3.428	0.001
<i>Eggs and egg products</i>	-0.276	-2.447	0.018
$R^2 = 0.376$			

TABLE 8:9

Multiple regression analysis for free oestradiol

Variables in Regression	Standard Regression Coefficient	T value	Significance of T value
<i>Meat and offal products</i>	0.502	4.538	0.000
<i>Eggs and egg products</i>	-0.267	-2.425	0.019
<i>Waist/hip ratio</i>	0.256	2.404	0.020
<i>% Energy from fat</i>	-0.355	-3.192	0.0024
<i>Alcohol</i>	-0.235	-2.209	0.032
$R^2 = 0.443$			

Dietary fibre intake emerged as an important predictor of both total testosterone and free testosterone concentrations in this study. Dietary fibre was able to explain 23% of the variation in testosterone levels in these subjects. Dietary cholesterol was also a predictive factor for both total and free testosterone. However, the waist/hip ratio correlated positively with free testosterone and was also an important factor in determining the concentrations of free testosterone, but not of total testosterone.

TABLE 8:10

Multiple regression analysis for testosterone

Variables in Regression	Standard Regression Coefficient	T value	Significance of T value
<i>Dietary fibre</i>	0.481	4.198	0.001
<i>Dietary cholesterol</i>	0.272	2.373	0.021
$R^2 = 0.306$			

TABLE 8:11

Multiple regression analysis for free testosterone

Variables in Regression	Standard Regression Coefficient	T value	Significance of T value
<i>Dietary fibre</i>	0.507	4.552	0.000
<i>Waist/hip ratio</i>	0.307	2.737	0.009
<i>Dietary cholesterol</i>	0.252	2.259	0.028
$R^2 = 0.363$			

SHBG levels in this study were negatively correlated with all anthropometric variables and significantly so with skinfold measurements, with the exception of biceps and triceps. However, multivariate analysis confirmed that upper body fat distribution was important in determining SHBG concentrations (Table 8:12). Twenty per cent of the variation in SHBG levels could be attributed to suprailiac skinfold measurement and waist/hip ratio, but suprailiac skinfold alone explained 13% of the variation.

TABLE 8:12

Multiple regression analysis for sex hormone binding globulin (SHBG)

Variables in Regression	Standard Regression Coefficient	T value	Significance of T value
<i>Suprailiac skinfold</i>	-0.280	-2.174	0.034
<i>Waist/hip ratio</i>	-0.278	-2.163	0.035
$R^2 = 0.202$			

8:4 Discussion

All the subjects in this study had normal menstrual cycles and none of them had been diagnosed as having any abnormalities in the breasts. Onset of early menarche is known to be related to better nutritional status and accelerated puberty, which may have been the case with the omnivores. Both vegetarian groups tended to have a later age of menarche than the omnivores. The Indians were lifelong vegetarians and almost all had spent their childhood in India or Africa. Most of the Caucasian vegetarians had adopted vegetarianism during the early years of childhood. This observation suggests that the delayed age of menarche in vegetarians requires confirmation.

SHBG concentrations were lower in the Indians than in the other groups. Multivariate analysis revealed a relationship with upper body obesity as measured by waist/hip ratio and suprailiac skinfold thickness. The low SHBG appears to be part of the constellation of metabolic abnormalities associated with upper body obesity. It has been argued that low SHBG concentrations may increase the relative proportion of free

testosterone to oestrogen because of the lower binding affinity of oestrogen to SHBG compared to testosterone. However, the results of this study do not confirm this hypothesis. In fact, the opposite seemed to be the case - upper body obesity was associated with a higher oestrogen/testosterone ratio.

Concentrations of circulating oestrogens did not differ significantly within groups. Relationships between plasma oestrogen levels, dietary and anthropometric variables were studied. Meat and offal products, as well as the percentage of energy derived from carbohydrates, were positively correlated with total oestradiol, whereas eggs and egg products were negatively correlated. Free oestradiol correlated positively with the percentage of energy from fat, meat and offal products and the waist/hip ratio, but negatively with eggs and egg products and alcohol intake. The relationship between meat and offal with both total and free oestradiol is of interest as Lee *et al.* (1991) reported an increase in the odds ratio for breast cancer with meat consumption in pre-menopausal women. The negative correlation between oestradiol and egg consumption is also of interest as Snowdon (1988) showed a clear dose-response relationship between egg consumption and ovarian cancer in Seventh-Day Adventists.

Total and free testosterone concentrations were greater in the Caucasian vegetarians than in the other groups. It is tempting to attribute this to a decrease in the conversion of androstenedione to oestrogen because of the lower proportion of body fat in Caucasian vegetarians. However, multivariate analysis suggested that dietary fibre intake and cholesterol intake were the best predictors of plasma testosterone concentration and that the waist/hip ratio was a predictor of free, but not of total testosterone concentrations. The strength of the relationship between fibre intake and testosterone concentrations could suggest a causal relationship. Certain types of dietary fibre are known to interfere with the absorption of oestrogen from the gut. For example, it is known that guar gum decreases the absorption of oral contraceptives (Todd *et al.*, 1990). It is possible, therefore, that dietary fibre may increase the catabolism of oestrogens by interfering with the enterohepatic circulation. This hypothesis could easily be tested by giving supplements of dietary fibre.

The results of this study do not show any marked effect of a vegetarian diet on free oestrogen concentration that would be consistent with decreased risk of breast cancer. The expression of oestrogenic activity is also determined by interaction of

oestrogen with receptors. Certain foods of plant origin, especially soya beans, contain phyto-oestrogens that compete with oestrogens for receptors. Depending on the biological activity of the phyto-oestrogens (Adlercreutz *et al.*, 1987), they may either enhance or decrease oestrogenic activity. Future studies should consider the possibility of oestrogenic material in food and use bio-assay to measure oestrogenic activity in plasma.

8:5 Summary

1. Sex hormone concentrations and SHBG levels were measured in Caucasian omnivores, Indian and Caucasian vegetarians.
2. There were no significant differences in the concentrations of oestradiol and free oestradiol between groups. Caucasian vegetarians had significantly greater levels of both total and free testosterone compared to the other groups. The ratio of free oestradiol to free testosterone was significantly lower in Caucasian vegetarians compared to the Indians.
3. Sex hormone binding globulin (SHBG) was significantly lower in the Indians compared to both groups of Caucasians.
4. Total oestradiol concentrations in plasma were related to the consumption of meat and offal products, eggs and carbohydrate, whereas variation in free oestradiol levels was related to the waist/hip ratio, percentage of energy from fat and alcohol intake.
5. Both the concentrations of free and total testosterone were related to the intakes of fibre and cholesterol and, additionally, free testosterone was also related to the waist/hip ratio.
6. None of the dietary factors significantly influenced SHBG levels in plasma. Suprailiac skinfold thickness and waist/hip ratios were negatively related to SHBG concentrations.
7. The results of this study do not show any marked effect of a vegetarian diet on sex hormone levels.

CHAPTER 9

FAECAL BILE ACIDS AND NEUTRAL STEROLS

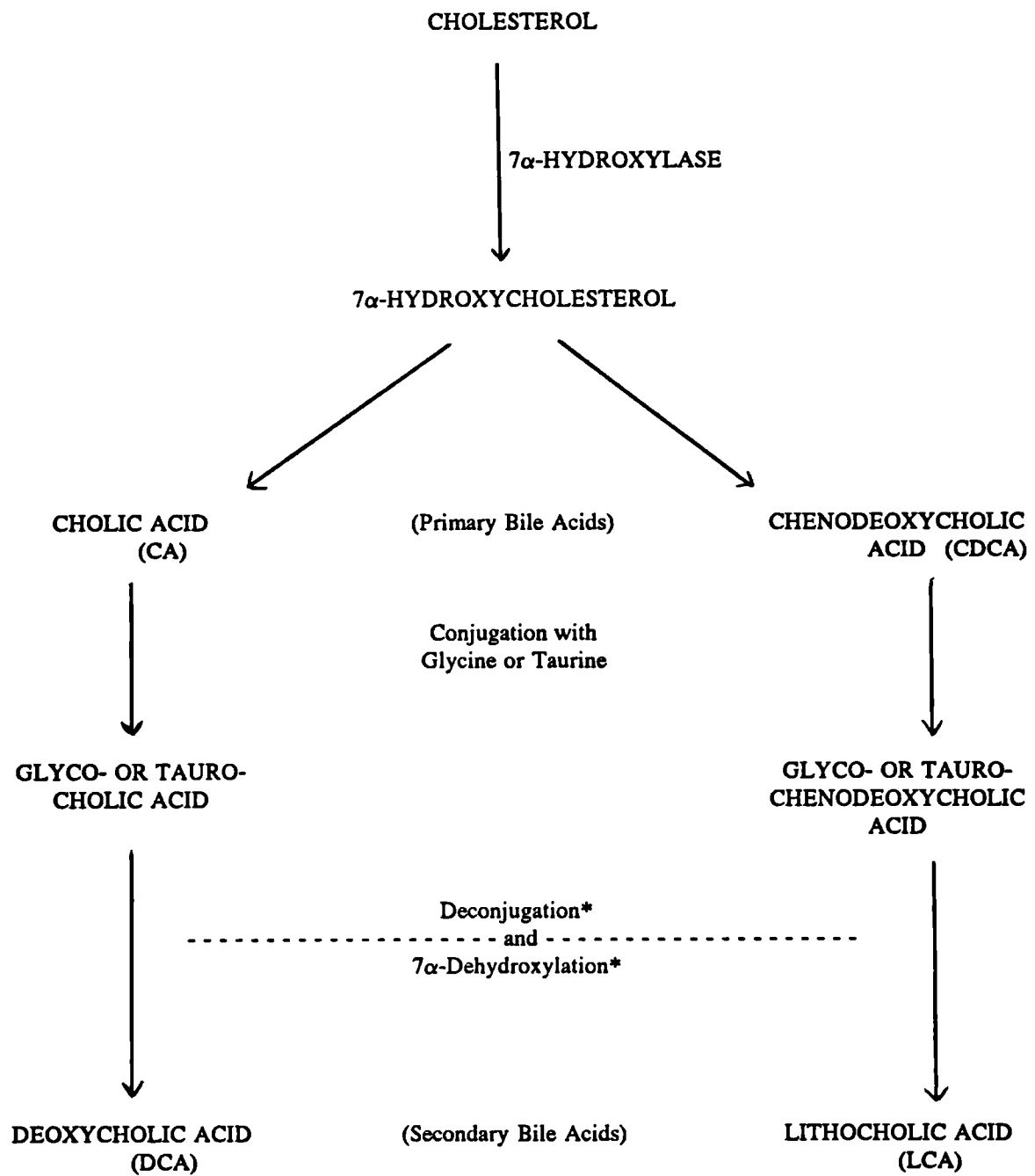
9.1: Introduction

Diet could play a role in the causation of colon cancer by providing carcinogens or co-carcinogens or by modifying intestinal secretions. One proposed mechanism is by modifying faecal steroid secretion. The intake of fat is a major determinant of bile acid secretion (Aries, 1969). Faecal steroid secretion is greater in persons from Western developed countries than in those from developing countries. Hill *et al.* (1971) suggested that bile acids and neutral sterols might be transformed into carcinogens or co-carcinogens by anaerobic bacteria in the gut. Further experimental studies also revealed the tumour promoting activity for certain sterols and bile acids and structural similarities between some sterols and polycyclic aromatic hydrocarbons, e.g. cholesterol and 3-methylchrysene (Nair, 1988).

Secondary bile acids are also known to be potent non-substrate inhibitors of glutathione sulfo-transferase (GST) activity *in vitro*. GST is involved in detoxification of exogenous carcinogens. Thus secondary bile acids may modify the endogenous host response to carcinogens. Lithocholic acid (LCA) was found to be the most potent inhibitor of rat hepatic GST in normal and phenobarbital-treated animals (Kessie and Nair, 1985). There is also evidence of a range of potentially toxic biological activities of specific secondary bile acids, especially LCA, on cellular and molecular processes such as mutagenicity, transforming activity, and DNA strand breakage. Case-control studies have shown that patients with colorectal polyps, ulcerative colitis and colo-rectal cancer have higher concentrations of faecal bile acids, particularly LCA (Thompson, 1985). However, subjects with familial polyposis who are at increased risk of colon cancer, did not have higher levels of faecal bile acids but these subjects may be unusually sensitive to low levels.

9:1:1: Excretion and Metabolism of Bile Acids

The major function of bile acids in the digestive tract is to facilitate the absorption of fat-soluble nutrients by acting as emulsifiers. The primary bile acids - cholic acid (CA) and chenodeoxycholic acid (CDCA) are synthesised in the liver from cholesterol. Cholic acid is found in the largest amount in the bile, but both CA and CDCA acids are formed from a common precursor derived from cholesterol (Fig. 9:1). Under normal circumstances in humans bile acids are synthesized by the liver at a rate of 200-500 mg/day. This rate is regulated to just replace the daily loss of bile acids in the faeces.



* Catalyzed by microbial enzymes

Figure 9:1

Biosynthesis and degradation of bile acids

The bile acids are the end products of cholesterol catabolism in the body. Because the tissues cannot break down the steroid nucleus, these compounds, together with cholesterol itself, which is also present in the bile, represent the only significant route for elimination of cholesterol from the body. Measurement of the faecal output of bile acids plus neutral sterols is therefore an accurate way to estimate the amount of cholesterol excreted from the body.

Entero-Hepatic Circulation of Bile Acids

Primary bile acids are synthesized from cholesterol by different pathways and from different pools of cholesterol: CDCA is synthesized mainly from dietary cholesterol, whilst CA is derived from endogenous cholesterol (Uchida *et al.*, 1982). The bile acids are then conjugated with amino acids (glycine or taurine) and then secreted in the bile. Bile is stored in the gall bladder where it is concentrated by the coupled removal of water and electrolytes. The hormone cholecystokinin causes gall bladder contraction and consequent discharge of bile into the duodenum via the bile duct. Cholecystokinin (CCK) is produced in response to a meal and so transfer of gastric contents from the stomach to the duodenum is linked to discharge of bile into the food. Fat is a potent stimulator of CCK release. Bile acids act as detergents and emulsify the dietary fat into micellar solution which is then transferred across the brush-border membrane of the small intestine for absorption. The absorption of fat is accompanied by the return of the bile acids into the gut lumen where they are available for further fat emulsification. The bile acids are removed by an active transport mechanism in the terminal ileum, which is an efficient system recovering both conjugated and free bile acids and supplements the less effective passive absorption systems present throughout the length of the small intestine. Following their active or passive resorption, the bile acids are returned to the liver via portal circulation where free bile acids are reconstituted to begin another enterohepatic cycle.

The human bile acid pool is 2-5 g and is larger in women than in men (Bennion *et al.*, 1978). It is inversely related to the frequency with which the bile acids are recycled (Northfeld and Hoffman, 1973) and the rate of gall bladder emptying (Duane and Hanson, 1978). The bile acid pool is usually recycled between 6 and 10 times per day; this is increased by dietary fat and decreased by dietary fibre (Meyer *et al.*, 1979). Despite the efficiency with which they are absorbed from the terminal ileum, a small

proportion (less than 5%) is lost into the large bowel. Some is recovered by passive diffusion from the colon, about 500 mg/day is lost in the faeces (Grundy *et al.*, 1965). The transfer of bile acids from portal blood is efficient but a small amount (less than 4 mg/day) escapes into the peripheral blood to be excreted in urine (Arborgh *et al.*, 1980).

The concentration of faecal bile acids depends on the amount of faeces and the efficiency of reabsorption, while the composition of the bile acids in faeces depends not only on the type of bile acids entering the colon but to the metabolic products of these bile acids by the action of gut bacterial flora.

9:1:2 Microbial Degradation of Bile Acids and Sterols

In healthy adults the luminal flora is complex and stable containing bacteria from many different genera but may be dominated by one particular organism. Bile acids may be degraded by intestinal organisms both *in vivo* and *in vitro* and this is not confined to specific bacteria, but are more frequently expressed by sub-populations of a number of organisms (Macdonald *et al.*, 1983). Therefore, while there may be only marginal differences in the general profiles of intestinal organisms between populations (Schwann *et al.*, 1982) or between individuals consuming defined diets (Goldberg *et al.*, 1977), there may be considerable differences in the bile acid degrading activity of the flora. Comparison of the faecal bile acid profiles of germ-free and conventional animals has confirmed that intestinal bacteria are involved in the degradation of these substrates. Germ-free rats secrete bile acids mainly in the conjugated form, whereas degraded products of bile acids form the major faecal bile acids in conventional animals (Thompson, 1985). Bile acids may be degraded *in vitro* by intestinal bacteria via a number of pathways.

1. **Desulphation:** Bile acid sulphates are degraded and it has been demonstrated that some Clostridia can degrade lithocholic-3 α -sulphate.
2. **Deconjugation:** Deconjugation activity is widely distributed throughout the intestinal flora including bacteroides, bifidobacteria, clostridia, enterococci, eubacteria, lactobacilli and streptococci. Some are capable of hydrolysing a range of conjugates, whereas others exhibit a specificity for either taurine or glycine conjugates and sometimes for particular bile acids. Both glycine and taurine

conjugates are rapidly degraded at physiological pH and very low levels are detected in faeces (Aries and Hill, 1970). The principal outcome of deconjugation is to release free bile acids for absorption or for further degradation by the gut bacteria.

3. **Dehydroxylation:** The bacteria that degrade 7 α -hydroxy bile acids represent only a small proportion of the colonic flora (Hylemon and Glass, 1983), but the reaction is carried out with a high degree of efficiency and are responsible for the conversion of primary bile acids in the colon to secondary bile acids - deoxycholic acid (DCA) and lithocholic acid (LCA). The enzyme cholanyl-7 α -dehydroxylase has been isolated in a range of anaerobic bacteria and is not produced *in vitro* when the pH is below 6.0, therefore the reaction may be inhibited by acidic pH in the colon. Although the bile acids in ileostomy effluent have not undergone dehydroxylation, those in faeces are more than 90% dehydroxylated (Hill and Aries, 1971). Dehydroxylation occurs principally in the proximal colon (Fadden *et al.*, 1984) and the products are then available for passive absorption from the colon. From assays of ileostomy and faecal bile acids (Fernandez, 1984, 1985) it can be calculated that approximately 100 mg is absorbed and a high proportion of this is DCA as it is more water-soluble than LCA and therefore easily diffuses through the gut wall.
4. **Oxido-reduction:** Cholic acid has hydroxyl groups at the 3, 7 and 12 positions and these are all oxidized by bacterial action to the oxo derivatives. The enzymes involved are all oxido-reductases, inducible and do not act oxidatively under strictly anaerobic conditions, therefore the gut environment favours their action as dehydrogenases. 7 β - and 6 β -hydroxysteroid dehydrogenases have been characterized and the activity of the latter is responsible for the epimerization of β -muricholic acid to ω -muricholic acid in rats and pigs.
5. **Nuclear Dehydrogenation:** Various enzymes are able to dehydrogenate the bile acid nucleus, the most important in quantitative terms is the 4-dehydrogenase which converts the 3-oxo-5 β -cholanoyl substrate to its 3-oxo-4-en analogue. A variety of species of clostridia present in the gut have this enzyme. The nuclear dehydrogenation reaction is reversible and following formation of the 3-oxo-4-

choloenoic acid the reduction step may yield either the 5 α or the 5 β configuration giving rise to the presence of 5 α bile acids (allo bile acids) in faeces. The detection of a range of such bile acids in human faeces is strong evidence that nuclear dehydrogenation occurs *in vivo* since no alternative source of this range of allo bile acids has been proposed (Wait *et al.*, 1985).

6. *The Degradation of Neutral Sterols:* Cholesterol is the major steroid substrate for bacterial metabolism in the human body, with 500-1000 mg per day passing through the colon and being excreted in faeces. In most persons less than 30% of the total cholesterol entering the colon escapes metabolism. The apparent co-carcinogenicity of cholesterol is still to be proven. In the gut, cholesterol is metabolized to coprostanol and coprostanone and these metabolic reactions could be considered as detoxification reactions removing the potential carcinogenic activity of the parent compound (Hill, 1986). Goddard and Hill (1972) demonstrated that bacteria could produce oestrogen analogues from cholesterol *in vitro* and *in vivo* (Goddard and Hill, 1974).

Microbial action in the gut through bacterial enzymes have also been implicated in generating mutagens, carcinogens and tumour promoters. Polycyclic aromatic hydrocarbons (PAHs) present in the environment and ingested or inhaled are detoxified in the liver, usually via hydroxylation followed by conjugation as the sulphate, glucouronide or glutathione derivatives and subsequently secreted in the bile. A wide range of bacterial species produce β -glucouronidase which could deconjugate PAHs and release them for enterohepatic circulation. Renwick and Drasar (1976) demonstrated that a high proportion of gut bacterial strains of human faecal organisms, especially clostridia, could dehydroxylate the hydroxy PAHs and so regenerate the parent carcinogen.

9:1:3 Diet and Faecal Flora

Although the faecal flora appears to be stable in individuals, the metabolic potential of the organisms may be affected by dietary changes. In some subjects reduction of meat content of their diet was associated with the excretion of a higher proportion of primary bile acids when compared to controls (Reddy *et al.*, 1975), indicating reduced microbial dehydroxylation. Individuals consuming an animal fat and protein enriched diet appear to carry, on average, more bacteroides and fewer enterococci

or similar anaerobes when compared to Africans or Indians consuming an essentially vegetarian diet (Drasar and Hill, 1974). Subjects living under essentially the same environmental conditions but consuming different diets, such as Seventh-Day Adventists in North America, have similar profiles of faecal organisms (Goldberg *et al.*, 1977; Hill *et al.*, 1982). Microbial bile acid deconjugation and oxidoreduction activity is generally distributed in these populations but those subjects consuming a mixed Western diet carry a higher concentration of organisms able to 7 α -dehydroxylate and dehydrogenate bile acids (Hill, 1975; Goddard *et al.*, 1975). Dietary supplements of wheat bran (Kay, 1982), bagasse (McLean *et al.*, 1977) or fat (Cummings *et al.*, 1978) are not associated with any significant changes in the profile of faecal organisms, the variation between individuals being greater than any due to apparent diet-related effects.

Only extreme dietary modification has been shown to cause variation in the faecal flora. Enterococci and lactobacilli decreased, whereas carriage of entero-bacteria increased in subjects consuming a chemically-defined soluble diet for a period of 10 days (Crowther *et al.*, 1973). However, only marginal changes in faecal flora were noted in subjects consuming boiled rice, vegetable or lean beef diets for three day periods (Moore and Holdeman, 1975). Although the relative proportions of organisms responsible for the metabolic degradation of bile acids and neutral sterols may not be influenced by dietary variation, faecal pH, Eh, water content and transit time are. Thus dietary variations can influence the environment of the large bowel which, in turn, can alter the microbial degradation of bile acids and neutral sterols.

9:1:4 Diet and Faecal Bile Acids

Inter-population studies have shown considerable differences in the faecal concentrations of bile acids (Chapter 1, Part II). In a detailed study of bile acid excretion in populations consuming different diets, Reddy *et al.* (1980) found that principally due to a higher intake of cereal products rural Finns excrete lower concentrations of bile acids compared to New York subjects consuming a mixed Western diet. Comparison of five North American groups with defined dietary practices demonstrated that Seventh Day Adventists, vegetarians, Japanese or Chinese subjects all excreted fewer bile acids than controls on a typical mixed diet (Reddy *et al.*, 1980). However, only in the vegetarian group was the degree of conversion to secondary bile acids reduced.

More specific information on the relationship between faecal bile acid excretion and the consumption of specific dietary items such as animal fat, protein and fibre has been observed in dietary intervention studies. Altering the consumption of fat is generally associated with a variation in faecal bile acid excretion, although the extent of the effect may depend on the composition of dietary fat. Decreasing fat intake is associated with reduced faecal bile acid concentration (Hill, 1971) and increasing dietary fat intake leads to an increase in bile acid loss (Cummings *et al.*, 1978). Varying the fat composition from saturated (cocoa butter) to polyunsaturated (corn oil) increased total bile acid excretion (Connor *et al.*, 1969), in particular that of deoxycholic acid. Sturdevant *et al.* (1973) reported an increased prevalence of gallstones in subjects receiving a diet high in linoleic acid ($\approx 15\%$ energy) compared with those receiving a diet high in saturated fat. However, less severe modification of fat intake may not lead to discernable differences. The intake of cholesterol may have a large effect on bile acid excretion (Thompson, 1985). This may be because of its direct effect on hepatic cholesterol synthesis.

In subjects transferred from a mixed Western diet to a meat free diet, bile acid excretion was unaltered, although the degradation of cholic acid was reduced (Reddy, 1981). Conversely, transferring from a normal Western diet to a meat supplemented diet is associated with an overall increase in faecal bile acid loss, mainly as secondary bile acids. However, modifying meat intake results in changes in fat intake. Cummings *et al.* (1979) varied protein intake in volunteers consuming a fat and fibre controlled diet and found that doubling protein intake resulted in only a marginal increase in faecal bile acid excretion. This may indicate that protein intake alone does not influence bile acid excretion.

The main hypothesis of the role of dietary fibre in bowel diseases suggests that increased fibre intake bulks the stool and dilutes the concentration of luminal contents. Wheat bran was associated with enhanced total loss of bile acids although the stool bulking effect of this fibre may cause a considerable reduction of faecal bile acid concentration (Kay, 1982). In contrast, oat bran supplementation increased the total loss of bile acids, but resulted in a significant increase in faecal bile acid concentration due to marginal stool bulking effect (Kirby *et al.*, 1981). Similar effects of guar gum supplementation have also been reported (Thompson *et al.*, 1985). Pectin supplementation

increased total daily bile acid loss by approximately one-third, but was not associated with an increase in faecal bile acid concentration (McLean *et al.*, 1977). The more soluble fibres do not significantly increase stool bulk and therefore are associated with an increase in faecal bile acid concentration. Soluble fibre such as oat bran, guar and pectin bind bile acids and increase excretion similar to resins such as cholestyramine.

9:1:5 Studies on Bile Acid Excretion in Vegetarians

Vegetarian diets invariably are high in fibre and lower in fat and consequently may alter the total quantity, concentration and the bile acid profile in faeces. Table 9:1 summarises the faecal excretion pattern of bile acids and neutral sterols in Seventh-Day Adventists with different dietary habits compared to the general population.

Excretion of secondary bile acids and neutral sterols was significantly greater in both non-vegetarian groups, whose daily fat intake was also greater. However, intakes of other nutrients such as fibre, cholesterol and protein were not reported, and the authors concluded that the low fat content of the vegan diet is responsible for the decrease in bile acid and neutral sterol excretion. Lower levels of faecal steroids were also found in British vegans (Aries *et al.*, 1971). So far only one study has reported faecal steroid excretion in Asians, the details of which are summarised in Table 9:2 (McKeigue *et al.*, 1989).

Both Asian male and female subjects (aged 26-65 years) excreted significantly lower concentrations of secondary bile acids and neutral sterols whereas the concentration of primary bile acids was greater than in the general population. There was no significant difference in the ratio of lithocholic to deoxycholic acids, which is thought to be a marker of colon cancer risk in case-control studies. Lithocholic acid has been shown to be a more potent colon tumour promoter in animals. In this study, both vegetarian and non-vegetarian Asians were included, as it was assumed that the diets of the two were similar. Such an assumption was questionable, as was the dietary methodology.

TABLE 9:1

Faecal bile acid and neutral sterol excretion in Seventh-Day Adventists

Faecal component (mg/g of lyophilized stools)	Seventh-Day Adventists			General Population Non- Vegetarians (n=31)
	Vegans (n=18)	Lacto-Ovo Vegetarians (n=40)	Non- Vegetarians (n=37)	
<u>Bile Acids</u>				
Cholic	0.21	0.17	0.19	0.13
Chenodeoxycholic	0.21	0.26	0.20	0.19
Deoxycholic	1.06 ^a	1.57 ^{a,b}	2.28 ^b	2.48 ^b
Lithocholic	0.69 ^a	1.67 ^b	1.72 ^b	3.24 ^c
Ratio of Secondary : Primary Bile Acids	6.96 ^a	14.04 ^b	17.27 ^b	39.24 ^c
<u>Neutral Sterols</u>				
Cholestanol	0.105 ^b	0.225 ^a	0.209 ^a	0.244 ^a
Coprostanol	1.81 ^c	3.920 ^b	5.44 ^{a,b}	8.37 ^b
Coprostanone	0.313 ^c	0.655 ^b	1.139 ^{a,b}	1.908 ^a
Total Neutral Sterols	2.515 ^c	7.384 ^b	9.789 ^{a,b}	12.23 ^a
Ratio of Cholesterol : Cholesterol Metabolites	0.358 ^{a,b}	12.42 ^{a,c}	5.971 ^a	0.599 ^a
Fat Intake (g/day)	64.3 ^c	72.1 ^{b,c}	99.6 ^a	89.6 ^b

^{a,b,c} Group means with dissimilar superscripts are significantly different from each other at $p = < 0.05$.

Sources: Nair *et al.* (1984) and Turjiman *et al.* (1984).

TABLE 9:2

Faecal bile acids and neutral sterols in Asians compared with the British

Faecal Sterols (mg/g dry weight)	ASIANS		BRITISH		Signifi- cance*
	Males (n=29)	Females (n=32)	Males (n=18)	Females (n=18)	
<u>Primary Bile Acids</u>					
<i>Chenodeoxycholic</i>	0.46	0.23	nil	nil	
<i>Cholic</i>	0.54	0.20	nil	nil	
<u>Secondary Bile Acids</u>					
<i>Lithocholic</i>	0.95	1.20	2.59	2.99	$p < 0.01$
<i>Deoxycholic</i>	1.91	2.00	3.45	4.40	$p < 0.05$
<i>Total Major Bile Acids</i>	3.86	3.64	6.02	7.39	$p < 0.05$
<i>Ratio of Lithocholic : Deoxycholic Acids</i>	0.65	0.89	0.95	0.85	NS
<u>Neutral Sterols</u>					
<i>Coprostanol</i>	3.08	3.78	15.24	20.01	$p < 0.001$
<i>Cholesterol + Coprastanone</i>	3.80	3.67	11.19	5.70	$p < 0.05$
<i>Total Neutral Animal Sterols</i>	6.88	7.45	26.54	26.61	$p < 0.001$
<i>% Total as Coprostanol</i>	40.1	45.4	59.5	74.9	$p < 0.001$

* Values adjusted for age and sex

Source: McKeigue *et al.* (1989).

This chapter compares the faecal steroid profile of pre-menopausal Indian vegetarians, Caucasian vegetarians and omnivores, and studies the relationship between diet and faecal steroid excretion.

9:2 Methods

Analytical methods are described in Chapter 2. Data that was not normally distributed was log transformed before statistical analysis. Differences between mean faecal steroid concentrations were tested by analysis of variance. Relationships between faecal bile acids and steroids and dietary factors were assessed by univariate and multivariate analyses.

9:3 Results

9:3:1 Faecal Characteristics

Faecal characteristics of vegetarian and omnivorous groups are given in Table 9:3. The mean faecal wet weight of the Indian vegetarians was significantly greater than the Caucasian omnivores. The faecal moisture content was greater in the Indians compared to both groups of Caucasians. However, there was no significant difference in the faecal dry weights between the groups. The faecal pH was lower in the Indian vegetarians.

TABLE 9:3

Faecal characteristics of the subjects

	CO (n=18)		IV (n=22)		CV (n=18)	
	Mean	SE	Mean	SE	Mean	SE
<i>Faecal wet weight/day</i>	117.2 ^a	13.45	185.7 ^b	20.31	159.6	19.7
<i>Faecal dry weight/day</i>	30.8	3.08	36.0	3.26	38.4	3.41
<i>Moisture (%)</i>	72.6 ^a	1.26	78.9 ^b	1.21	74.6 ^a	1.05
<i>pH</i>	6.7 ^a	0.12	6.2 ^b	0.13	6.5 ^a	0.13

9:3:2 Faecal Bile Acids

There was a considerable between-subject variation in faecal bile acid concentrations. A higher proportion of Indian subjects excreted detectable amounts of primary bile acids (cholic and chenodeoxycholic) than the Caucasian omnivores (Table 9:4). Secondary bile acids were detected in all the Caucasian subjects and in most of the Indians.

TABLE 9:4

Number of subjects in whom faecal bile acids were detected (percentages in parentheses)

Bile Acids	CO (n=18)	IV (n=22)	CV (n=18)
<i>Cholic acid</i>	1 (5.6)	6 (27.3)	2 (11.1)
<i>Chenodeoxycholic acid</i>	1 (5.6)	6 (27.3)	3 (16.7)
<i>Lithocholic acid</i>	18 (100)	20 (90.9)	18 (100)
<i>Deoxycholic acid</i>	18 (100)	20 (90.9)	18 (100)

The concentrations of total major bile acids (Σ LCA + DCA + CA + CDCA) were lower in Caucasian vegetarians than in the Indians and omnivores (Table 9:5). Total free bile acid excretion (Σ total major + total minor bile acids) did not differ between groups. However, concentrations of total free bile acids on dry weight basis were lower in Caucasian vegetarians compared to the Indians and omnivores, while the concentrations on wet weight basis were lower in both groups of vegetarians compared to the omnivores. Total conjugated bile acids were greater in the Caucasian omnivores than the vegetarians, both on a wet weight and dry weight basis. Bile acids are conjugated with either glycine or taurine. It was not possible to distinguish with which amino acid they were conjugated.

The concentrations of primary bile acids were not significantly different between the groups. LCA concentrations were significantly lower in the Indian vegetarians both on a wet weight and dry weight basis. DCA concentrations were also lower on a wet weight basis.

TABLE 9:5 Faecal bile acid concentrations

	Caucasian Omnivores (n=18)		Indian Vegetarians (n=22)		Caucasian Vegetarians (n=18)		P Value of F
	Geometric Mean	95% Confidence Limits	Geometric Mean	95% Confidence Limits	Geometric Mean	95% Confidence Limits	
<u>Total Major Bile Acids</u> mg/g of dry weight mg/g of wet weight mg/day	5.58 ^a 1.50 ^a 151.2	4.19 - 7.44 1.15 - 1.96 98.7 - 231.6	4.67 ^a 0.96 ^b 155.6	3.79 - 5.75 0.75 - 1.23 115.7 - 209.2	3.08 ^b 0.77 ^b 109.9	2.75 - 4.29 0.56 - 1.06 71.8 - 168.0	0.009 0.004 0.333
<u>Total Minor Bile Acids</u> mg/g of dry weight mg/g of wet weight mg/day	0.81 ^a 0.22 ^a 21.8	0.56 - 1.15 0.14 - 0.33 15.6 - 30.6	0.40 ^b 0.08 ^b 13.4	0.26 - 0.62 0.05 - 0.14 8.3 - 21.6	0.50 0.13 17.9	0.37 - 0.68 0.09 - 0.17 12.2 - 26.4	0.029 0.007 0.214
<u>Total Free Bile Acids</u> mg/g of dry weight mg/g of wet weight mg/day	6.79 ^a 1.82 ^a 183.8	5.43 - 8.48 1.46 - 2.27 128.3 - 263.5	5.34 ^a 1.10 ^b 177.9	4.45 - 6.40 0.87 - 1.39 135.1 - 234.4	3.72 ^b 0.93 ^b 132.6	2.76 - 5.01 0.69 - 1.25 88.9 - 197.9	0.002 0.0008 0.318
<u>Total Conjugated Bile Acids</u> mg/g of dry weight mg/g of wet weight mg/day	0.66 ^a 0.18 ^a 17.9	0.50 - 0.87 0.13 - 0.24 11.5 - 27.7	0.53 0.11 ^b 17.6	0.41 - 0.68 0.08 - 0.15 12.9 - 24.1	0.40 ^b 0.10 ^b 14.1	0.33 - 0.47 0.08 - 0.12 10.6 - 18.9	0.015 0.012 0.552
<u>Cholic Acid</u> µg/g of dry weight µg/g of wet weight µg/day	1.6 1.5 1.9	0.6 - 4.0 0.7 - 3.3 0.5 - 7.2	6.2 3.9 16.7	1.5 - 25.1 1.3 - 11.1 2.0 - 137.7	1.8 1.5 2.8	0.8 - 4.2 0.8 - 2.6 0.6 - 12.9	0.142 0.166 0.146

	Caucasian Omnivores (n=18)			Indian Vegetarians (n=22)			Caucasian Vegetarians (n=18)			P Value of F
	Geometric Mean	95% Confidence Limits		Geometric Mean	95% Confidence Limits		Geometric Mean	95% Confidence Limits		
<u>Cholic Acid</u>										
µg/g of dry weight	1.6	0.6 - 4.0		6.2	1.5 - 25.1		1.8	0.8 - 4.2		0.142
µg/g of wet weight	1.5	0.7 - 3.3		3.9	1.3 - 11.1		1.5	0.8 - 2.6		0.166
µg/day	1.9	0.5 - 7.2		16.7	2.0 - 137.7		2.8	0.6 - 12.9		0.146
<u>Chenodeoxycholic Acid</u>										
µg/g of dry weight	1.6	0.6 - 4.1		6.9	1.6 - 29.4		2.6	0.9 - 7.9		0.190
µg/g of wet weight	1.5	0.7 - 3.4		4.2	1.4 - 12.5		1.2	0.9 - 4.4		0.224
µg/day	1.9	0.5 - 7.3		19.1	2.1 - 169.7		4.9	0.8 - 30.9		0.190
<u>Lithocholic Acid</u>										
mg/g of dry weight	2.61 ^a	2.07 - 3.30		0.85 ^b	0.32 - 2.30		1.45	1.14 - 1.86		0.058
mg/g of wet weight	0.70 ^a	0.57 - 0.87		0.33 ^b	0.25 - 0.49		0.36	0.28 - 0.47		0.009
mg/g day	70.8	48.1 - 104.2		55.8	41.3 - 75.5		51.8	36.5 - 73.7		0.162
<u>Deoxycholic Acid</u>										
mg/g of dry weight	2.72	1.98 - 3.74		2.48	1.91 - 3.24		1.44	0.91 - 2.26		0.264
mg/g of wet weight	0.73 ^a	0.55 - 0.97		0.22 ^b	0.12 - 0.69		0.36	0.23 - 0.56		0.094
mg/day	73.8	47.1 - 115.5		29.6	6.5 - 134.4		51.3	30.6 - 86.1		0.435

^{a,b} Group means with dissimilar superscripts are significantly different from each other at $p < 0.05$.

Thompson *et al.* (1985) proposed the ratio of LCA : DCA and the bile acid index (ratio(Σ LCA + DCA)) as indicators of risk of colon cancer. The ratio of LCA : DCA was lower in the Indian vegetarians than in either the Caucasian omnivores or vegetarians (Table 9:6). The bile acid index was lower in both the vegetarian groups.

TABLE 9:6

Measures of colon cancer risk indices in the subjects

	CO (n=18)		IV (n=22)		CV (n=18)	
	Mean	SE	Mean	SE	Mean	SE
<i>Ratio of Lithocholic : Deoxycholic acid</i>	1.0 ^a	0.07	0.68 ^b	0.090	1.18 ^a	0.163
<i>Bile Acid Index [Ratio (ΣLCA + DCA)]</i>	6.89 ^a	0.641	3.76 ^b	0.555	4.15 ^b	0.458

^{a,b} Group means with dissimilar superscripts are significantly different from each other at $p < 0.05$.

9:3:3 Faecal Neutral Sterols

Faecal neutral sterol excretion is given in Table 9:7. Total faecal neutral sterol concentrations were lower in both groups of vegetarians compared with omnivores, both on a wet and dry weight basis. This can be attributed to a lower concentration of animal sterols. Coprostanol, which is a bacterial degradation product of cholesterol, accounted for a higher proportion of total animal sterols in the Caucasian subjects compared with the Indian vegetarians (Table 9:8). The faecal concentrations and daily excretion of coprostanol was significantly lower in the Indian vegetarians. Cholesterol and coprostanone concentrations were similar in Caucasian omnivores and Indian vegetarians. No differences in cholesterol sulphate concentration were noted on a dry weight basis.

TABLE 9:7 Faecal neutral steroid concentrations

Neutral Sterols in Faeces	Caucasian Omnivores (n=18)		Indian Vegetarians (n=22)		Caucasian Vegetarians (n=18)		P Value of F
	Geometric Mean	95% Confidence Interval	Geometric Mean	95% Confidence Interval	Geometric Mean	95% Confidence Interval	
<u>Total Animal Sterols</u>							
mg/g of dry weight	26.3 ^a	21.1 - 32.8	14.6 ^b	12.0 - 17.9	13.3 ^b	10.0 - 17.8	0.0002
mg/g of wet weight	7.1 ^a	5.6 - 8.9	3.0 ^b	2.3 - 3.9	3.3 ^b	2.4 - 4.5	0.0000
mg/day	713.3 ^a	523.8 - 971.4	488.0 ^b	394.3 - 603.9	476.2	341.8 - 663.3	0.072
<u>Total Plant Sterols</u>							
mg/g of dry weight	15.2	13.4 - 17.2	13.4	11.5 - 15.7	13.1	10.9 - 15.8	0.355
mg/g of wet weight	4.1 ^a	3.4 - 4.9	2.8 ^b	2.2 - 3.4	3.3	2.6 - 4.1	0.024
mg/day	411.8	313.2 - 541.5	447.9	354.1 - 566.5	467.5	359.3 - 608.3	0.770
<u>Total Neutral Steroids</u>							
mg/g of dry weight	41.9 ^a	35.1 - 50.0	28.5 ^b	24.2 - 33.5	26.7 ^b	21.2 - 33.6	0.002
mg/g of wet weight	11.3 ^a	9.2 - 13.8	5.8 ^b	4.6 - 7.4	6.7 ^b	5.1 - 8.7	0.0003
mg/day	1134.2	849.4 - 1515.0	949.7	771.2 - 1169.8	951.6	710.7 - 1273.2	0.522

Neutral Sterols in Faeces	Caucasian Omnivores (n=18)		Indian Vegetarians (n=22)		Caucasian Vegetarians (n=18)		P Value of F
	Geometric Mean	95% Confidence Interval	Geometric Mean	95% Confidence Interval	Geometric Mean	95% Confidence Interval	
<u>Coprastranol</u> mg/g of dry weight mg/g of wet weight mg/day	18.4 ^a 5.0 ^a 498.8 ^a	14.4 - 23.6 3.8 - 6.5 352.4 - 706.2	2.7 ^b 0.6 ^b 90.8 ^b	1.2 - 6.2 0.3 - 1.3 41.4 - 199.0	6.8 ^c 1.7 ^c 243.2 ^c	4.2 - 11.0 1.0 - 2.8 149.1 - 396.8	0.0001 0.0000 0.0004
<u>Cholesterol + Coprastranone</u> mg/g of dry weight mg/g of wet weight mg/day	7.0 ^a 1.9 ^a 190.7	5.3 - 9.3 1.5 - 2.5 140.6 - 258.5	7.1 ^a 1.5 235.2	5.3 - 9.4 1.1 - 2.0 168.3 - 328.9	4.5 ^b 1.1 ^b 161.4	3.1 - 6.6 0.8 - 1.7 101.9 - 255.6	0.068 0.077 0.311
<u>Cholesterol Sulphate</u> µg/g of dry weight µg/g of wet weight µg/g day	206.5 55.5 ^a 5.6	148.8 - 286.7 39.6 - 77.8 3.6 - 8.6	150.3 30.8 ^b 5.0	121.7 - 185.6 24.3 - 39.1 3.7 - 6.7	211.2 52.8 ^c 7.5	175.4 - 254.3 42.4 - 65.8 5.9 - 9.7	0.069 0.002 0.177

^{a,b,c} Group means with dissimilar superscripts are significantly different from each other at $p < 0.05$.

TABLE 9:8

Proportions of faecal sterols

	CO (n=18)		IV (n=22)		CV (n=18)	
	Mean	SE	Mean	SE	Mean	SE
% Coprostanol in total animal sterols	71.0 ^a	2.84	42.0 ^b	7.11	60.8 ^a	5.76
% Plant sterols in total neutral sterols	36.8 ^a	1.48	48.0 ^b	1.83	49.5 ^b	1.51

^{a,b} Group means with dissimilar superscripts are significantly different from each other at $p < 0.05$.

9:3:4 Relationship between Diet and Faecal Variables

Multivariate analysis was conducted to assess relationships between faecal variables and dietary factors. Cereals and vegetables were associated positively with faecal dry weight, while nuts and fish showed a negative association and the four food groups explained 30% of the variation in faecal dry weights between groups. Among the nutrients, starch intake accounted for 14% of the variation in faecal dry weights (Table 9:9).

TABLE 9:9

Multiple regression for faecal dry weight

Variables in Regression	Regression Coefficient	T Value	Significance of T
<u>Foods Consumed</u>			
Cereals	0.252	2.061	0.044
Vegetables	0.274	2.291	0.026
Nuts	-0.352	-2.939	0.005
Fish	-0.299	-2.423	0.019
$R^2 = 0.301$			
<u>Nutrient Intake/Day</u>			
Starch	0.377	2.99	0.004
$R^2 = 0.142$			

Differences in the concentrations of LCA and DCA between the groups could be explained solely by the proportion of energy intake from saturated fatty acids (Table 9:10). None of the individual foods was predictive of the concentrations of LCA and DCA. No significant correlations were found between dietary variables and the ratio of LCA : DCA. However, a significant correlation between faecal pH and this ratio ($r = 0.31$, $p=0.009$) was present.

TABLE 9:10

Multiple regression for secondary bile acids (mg/g of dry weight)

Variables in Regression	Regression Coefficient	T Value	Significance of T
<u><i>Lithocholic Acid</i></u>			
% Energy from saturated fatty acids	0.374	2.962	0.005
$R^2 = 0.140$			
<u><i>Deoxycholic Acid</i></u>			
% Energy from saturated fatty acids	0.340	2.658	0.010
$R^2 = 0.116$			

The concentrations of free bile acids are believed to be important in colon carcinogenesis. Pulses were negatively associated with the concentrations of free bile acids in these subjects and 20% of the variation between groups could be attributed to the intake of pulses. Among the nutrients, fibre and energy intakes were significant predictors of faecal free bile acid concentrations in these subjects. This corroborates the negative association of fibre with faecal bile acid concentrations (Table 9:11).

TABLE 9:11

Multiple regression for total free bile acids (mg/g of dry weight)

Variables in Regression	Regression Coefficient	T Value	Significance of T
<u><i>Foods Consumed</i></u>			
<i>Pulses</i>	-0.448	-3.685	0.0005
$R^2 = 0.201$			
<u><i>Nutrient Intake/Day</i></u>			
<i>Fibre</i>	-0.790	-6.031	0.000
<i>Energy</i>	0.425	3.243	0.002
$R^2 = 0.408$			

Meat and offal products contributed a significant amount of cholesterol in the diet of the omnivores and were positively related to the concentrations of animal sterols in faeces (Table 9:12). Pulses were negatively associated with the faecal concentration of animal sterols. Dietary cholesterol and fibre intakes could explain 37.8% of the variation in animal sterol concentrations in faeces. Coprostanol, which is a degradation product of cholesterol, was strongly associated with dietary cholesterol (Table 9:13). The proportion of energy from polyunsaturated fatty acids was negatively associated with coprostanol concentrations in faeces. Twenty-six per cent of the variation between groups could be attributed to the intakes of cholesterol and polyunsaturated fatty acids.

TABLE 9:12

Multiple regression for total animal sterols (mg/g of dry weight)

Variables in Regression	Regression Coefficient	T Value	Significance of T
<u><i>Foods Consumed</i></u>			
<i>Meat and offal products</i>	0.351	2.836	0.007
<i>Pulses</i>	-0.277	-2.242	0.029
$R^2 = 0.255$			
<u><i>Nutrient Intake</i></u>			
<i>Dietary cholesterol</i>	0.461	4.254	0.0001
<i>Fibre</i>	-0.402	-3.705	0.0005
$R^2 = 0.378$			

TABLE 9:13

Multiple regression for coprostanol (mg/g of dry weight)

Variables in Regression	Regression Coefficient	T Value	Significance of T
<u><i>Foods Consumed</i></u>			
<i>Nuts</i>	-0.325	-2.658	0.010
<i>Meat and offal products</i>	0.310	2.531	0.014
$R^2 = 0.249$			
<u><i>Nutrient Intake/Day</i></u>			
<i>Dietary cholesterol</i>	0.379	3.097	0.003
<i>% Energy from polyunsaturated fatty acids</i>	-0.257	-2.098	0.041
$R^2 = 0.259$			

The proportion of coprastanol in faeces is indicative of cholesterol degradation in the intestinal lumen. Nuts were negatively associated with the proportion of coprastanol and 9% of the variation between groups was explained by the intake of nuts (Table 9:14). The sulphur to nitrogen (S/N) ratio is an indicator of quality of protein in the diet. Vegetable proteins are usually limited in their sulphur-amino acid content with the exception of soya protein, and thus have a lower S/N ratio. Caucasian omnivores had a significantly greater S/N ratio due to the inclusion of animal protein in the diet compared to both groups of vegetarians (Table 9:15). Caucasian vegetarians also had greater S/N ratios compared to the Indians. This may be because of the popularity of soya products with this group and their higher consumption of eggs. The S/N ratio was strongly associated with the proportion of coprastanol in faeces and accounted for 18% of the variation in cholesterol degradation.

TABLE 9:14

Multiple regression for percentage of coprastanol

Variables in Regression	Regression Coefficient	T Value	Significance of T
<u><i>Foods Consumed</i></u>			
<i>Nuts</i>	-0.299	-2.302	0.025
$R^2 = 0.089$			
<u><i>Nutrient Intake/Day</i></u>			
<i>S/N ratio</i>	0.429	3.491	0.001
$R^2 = 0.184$			

TABLE 9:15

Sulphur/nitrogen (S/N) ratio of the diets

	Mean	SE	95% CI
<i>Caucasian omnivores (n=22)</i>	51.8 ^a	1.51	48.7 - 55.0
<i>Indian vegetarians (n=21)</i>	33.7 ^b	2.05	29.4 - 37.9
<i>Caucasian vegetarians (n=18)</i>	41.5 ^c	1.68	38.0 - 45.1

a,b,c Values with dissimilar superscripts are significantly different from each other.

9:4 Discussion

The intra-luminal concentrations and metabolic transformation of bile acids and neutral sterols are believed to alter colon cancer risk in both humans and animals. Faecal characteristics also influence the concentrations of bile acids which in turn are influenced by diet, particularly dietary fibre and fat. Dietary fibre intakes were twice as high in the Caucasian vegetarians compared to the omnivores and Indians. The total faecal output of bile acids per day did not differ between groups, but the faecal concentrations on both dry and wet weight basis were lower in both vegetarian groups. Moreover a lower proportion of bile acids was present, particularly in the Indian vegetarians. Significantly lower concentrations of LCA in the Indians could suggest that they may be at lower risk of colon cancer. However, the Caucasian vegetarians also had significantly lower concentrations of total free and conjugated bile acids on both wet and dry weight bases. This suggests that the concentrations of bile acids in the lumen might be more important rather than the total output. Faecal characteristics such as faecal bulk and moisture content reduce the level of cellular contact of these potential co-carcinogens within the intestinal lumen, which might imply a protective role for complex carbohydrates.

The transformation of primary bile acids to secondary bile acids is dependent on the environment in the intestinal lumen. The faecal pH of the Indian vegetarians was more acidic compared to the Caucasians, which may have resulted in fewer primary bile acids being converted to secondary bile acids. A change to a vegan diet has been shown to decrease the faecal pH (Van Dokkum *et al.*, 1983) and both cholesterol and bile acid degradation are acid-sensitive. Suppression of faecal steroid conversion has been reported in both English vegans and in lacto-vegetarian Seventh Day Adventists (Aries *et al.*, 1971; Nair *et al.*, 1984; Turjiman *et al.*, 1984). Multivariate analysis revealed that pulses were the major predictors of faecal pH leading to acidic pH. Pulses were popular with the vegetarians compared to the non-vegetarians. It could be hypothesised that the complex carbohydrates provided by the pulses were fermented by bacteria to short chain fatty acids which decreased the faecal pH. It is notable that untransformed primary bile acids were detected in nine vegetarians (6 Indians and 3 Caucasians) compared to only one omnivore.

Previous studies have shown that the gut flora is quite stable in individuals but their metabolic potential may be affected by diet, especially animal protein, dietary fibre and fat. Both the number and metabolic activity of the various micro-organisms in the gut may be affected (Hill, 1976; Indra *et al.*, 1980). Multivariate analyses revealed a considerable influence of S/N ratio on the conversion of cholesterol to coprostanol. The rate of degradation of cholesterol to coprostanol was significantly greater in the omnivores and so was the S/N ratio. The S/N ratio could be regarded as an indicator of animal protein in the omnivorous diet. This may explain the consistent association of animal protein consumption and colon cancer risk observed in epidemiological studies. The higher dietary S/N ratios of the Caucasian vegetarian diets compared to the Indian diet could be attributed to a greater consumption of eggs and soya products by the Caucasian vegetarians.

Dietary fat is believed to increase the output of bile acids. Multivariate analyses of the data showed dietary fat intake, particularly saturated fat, as the best predictor of faecal bile acid concentrations as well as the total output of bile acids. Cholesterol and its metabolites in faeces are also implicated in colon cancer. Excretion of total animal sterols was greater in the omnivores and consistent with their higher intakes of dietary cholesterol. Also, a higher proportion of faecal animal sterols was found in the omnivores. The intake of plant sterols was not measured in this study but there was little difference in the excretion of plant sterols between the groups. However other studies (Nair *et al.*, 1984) reported that vegetarians and vegans have higher intakes of plant sterols such as β -sitosterol and stigmasterol.

A vegetarian diet usually contains greater amounts of protective dietary factors such as dietary fibre, vitamin C, β -carotene, etc. The presence of these factors may in itself abrogate the effects of co-carcinogens in the colon. Consumption of cruciferous vegetables in particular has been associated with decreased risk of colon cancer. In the present study both groups of vegetarians had lower bile acid index, which may indicate that they are at a lower risk compared to the omnivores. The LCA : DCA ratio was significantly lower in the Indians compared to both groups of Caucasians. McKeigue *et al.* (1989) also reported similar LCA : DCA ratios in men, but not in women. The lower ratio in the Indians is consistent with the low mortality from colon cancer found in this group. The bile acid and neutral sterol profile appears to be altered in vegetarians and

particularly in Indian vegetarians. The underlying mechanisms are still unclear but the results of the present study show that vegetarian diets alter the output of bile acids and thereby may influence the risk of colon cancer.

9:5 Summary

1. Faecal bulk, pH, moisture content and concentrations of sterols and bile acids were measured in 22 Indian vegetarian, 18 Caucasian omnivore and 18 Caucasian vegetarian women (aged 25-40 years).
2. Faecal bulk and moisture content were greatest and pH lowest in the Indian vegetarians. Total faecal bile acid and neutral sterol concentrations were lower in both Indian and Caucasian vegetarians.
3. The ratio of lithocholic/deoxycholic acid and that of coprostanol/total animal sterols was lower in the Indian vegetarians compared with Caucasian vegetarians and omnivores.
4. Multivariate analysis revealed statistically significant associations between: the proportion of energy derived from saturated fatty acid and faecal secondary bile acid concentrations; the intake of cholesterol and faecal neutral sterol concentrations; the sulphur/nitrogen ratio and the ratio of coprostanol/total animal sterols. There was a significant positive relationship between faecal pH and the ratio of lithocholic/deoxycholic acid.
5. The lower faecal sterol and bile acid concentrations and the lower proportion of secondary metabolites of bile acids and faecal sterols in the Indians are consistent with a decreased risk of colon cancer.

CHAPTER 10

FATTY ACID COMPOSITION OF CORD PLASMA AND UMBILICAL ARTERIES

10:1 Introduction

In Chapter 6 lower proportions of docosahexaenoic acid (22:6n-3) were found in the plasma phospholipids of Indian vegetarian women compared with controls. Docosahexaenoic acid (22:6n-3; DHA) is believed to play an important role in the development of the retina and the central nervous system (Crawford and Sinclair, 1972; Neuringer *et al.*, 1984). When α -linolenic acid is absent from the diet, docosapentaenoic acid (22:5n-6) replaced DHA in brain and retinal lipids. The ratio of linoleic to linolenic acid in the diet is an important predictor of the relative proportions of 22:5n-6/22:6n-3 in brain and retinal lipids (Mohrhauer and Holman, 1963; Sanders and Naismith, 1979; Sanders *et al.*, 1984). Liu *et al.* (1987) suggested that the preterm infant may have a dietary requirement for docosahexaenoic acid. Uauy *et al.* (1990) have shown that preterm infants fed diets devoid of α -linolenic acid have abnormal electroretinograms compared with those given diets containing DHA. As brain growth occurs mainly *in utero*, the fetus is dependent upon a maternal supply of α -linolenic acid and docosahexaenoic acid.

It has been argued that the fetus obtains DHA for its developing brain by a combination of preferential uptake by the placenta and fetal brain (Crawford *et al.*, 1976) and also by the high capacity of the fetal brain to convert α -linolenic acid to DHA (Sanders and Naismith, 1980). Cord plasma contains higher proportions of arachidonic acid and DHA than maternal plasma (Robertson and Sprecher, 1968) and the proportions of linoleic and α -linolenic acid were even lower. Hornstra *et al.* (1989) found very low levels of linoleic acid in cord arterial lipids but reported the presence of arachidonic acid (20:4n-6), docosapentaenoic acid (22:5n-6), DHA and eicosatrienoic acid (20:3n-9). The latter is sometimes regarded as the hallmark of an inadequate intake of linoleic acid in other tissues.

Crawford *et al.* (1989) have suggested that low birth weight may be related to an inadequate supply of essential fatty acids. It was claimed that the ratio of 20:3n-6/20:4n-6 was high in low birth weight infants. However, the statistical analysis in this study did not adjust for other confounding factors. Stammers *et al.* (1989) reported higher levels of arachidonic acid in the free fatty acid fraction of cord plasma from infants of Hindu vegetarian women. No differences in the concentration of docosahexaenoic acid were noted.

Low birth weight (<2500 g) is more common in babies born to mothers of Indian origin. Within the Indian sub-population, Hindu women give birth to smaller babies than Muslim women (McFadyan *et al.*, 1984). The difference in birth weight cannot be explained solely by differences in gestational age or maternal stature. The duration of gestation amongst the Gujarati population, which is predominantly Hindu, is approximately 4 days shorter compared with the indigenous UK population.

The maternity unit at Northwick Park Hospital serves the large multi-ethnic population of North London. Computerised information on parity, complications of pregnancy, smoking habits, religion and dietary habits (vegetarian/non-vegetarian) was available on the patients booked in for maternity care from approximately the first trimester of pregnancy. The opportunity existed therefore to obtain samples of umbilical cord and blood from a series of Asian vegetarian deliveries and from Caucasian controls in order to determine the fatty acid composition of plasma phospholipids and cord arterial phospholipids.

10:2 Methods

10:2:1 Subjects

Cord blood samples and umbilical cords were collected from 146 deliveries at Northwick Park Hospital between September and November 1990. Cords were washed in saline and blood was anti-coagulated with EDTA (1mg/ml) and plasma separated. Plasma and cords were stored at -20°C until analysed 3-4 months later. Samples were identified by name and hospital number and information on the pregnancy and birth were extracted from the computerised records. Samples were obtained from 48 Hindu vegetarians and 98 Caucasian controls. Samples from 32 Hindu vegetarians were compared with those from 32 non-vegetarian Caucasians matched for age, parity, gender of newborn and gestational age.

10:2:2 Analytical methods

The cord was defrosted and the artery dissected out. Lipids were extracted from the arteries and plasma with 20 vol. ice-cold chloroform:methanol mixture (1:1 by volume) containing butylated hydroxytoluene (50 mg/litre). Diheptadecanoyl-phosphatidylcholine was added as an internal standard to the plasma extract. Phospholipids were extracted from the total lipid extracts by the use of amino-bonded

SepPak cartridges (Kaluzny *et al.*, 1985). Acetyl chloride (0.2 ml) was added to the methanol eluate (4 ml) from the amino bonded cartridge and heated in a sealed tube for 1 hour. On cooling 5 ml of 60 g sodium carbonate/l was added and 200 μ l of toluene. The sample was mixed and centrifuged and 100 μ l of the toluene supernatant was taken for analysis by gas-liquid chromatography. Gas-liquid chromatography of methyl esters was carried out on a 25 mm x 0.2 mm internal diameter vitreous silica column coated with CpCil 88 (Chromopak Ltd, London) using hydrogen as the carrier gas. Chromatograms were integrated using a Shimadzu integrator. Fatty acid methyl esters were identified by comparison to standards obtained from Sigma (Poole, Dorset) and the use of secondary reference standards.

10:2:3 Statistical Analysis

Comparisons between groups were made by analysis of variance. Ninety-five per cent confidence intervals were calculated by multiplying the estimate of standard error by 2. The standard deviation was calculated as the square root of the residual mean square. Frequencies were compared using a Chi-squared test or where cell numbers were small Fisher's Exact Probability test. Comparisons for fatty acid analysis were made using a paired t-test.

10:3 Results

Table 10:1 shows the characteristics of the total sample. Maternal age and parity were similar in both groups. In contrast to the Indian women, a significant proportion of the control population was single/unmarried and approximately one-third were smokers. The prevalence of anaemia during pregnancy was slightly higher in the Indians and that of hypertension lower. Overall complications of pregnancy were less common in the Indian women but this did not achieve statistical significance. The Indian women were shorter and lighter (at booking for the maternity clinic) than the controls.

TABLE 10:1**Maternal characteristics of 48 Indians and 96 Caucasians**

	Indian vegetarians (n=48)	Controls (n=98)	P Value
<i>Maternal age (yrs)</i> <i>Mean (95% Confidence Interval)</i>	29 (27.9-30.3)	28.4 (27.2-29.5)	NS
<i>Maternal height (cm)</i> <i>Mean (95% CI)</i>	157 (155.9-158.9)	163 (161.8-164.4)	<0.01
<i>Maternal weight (kg)</i> <i>Mean (95% CI)</i>	58.8 (56.1-61.5)	62.6 (60.5-64.7)	0.032
<i>% smokers</i>	2.1	34.1	<0.01
<i>% Primiparous</i>	43.8	47.8	NS
<i>% Married/Divorced/ Widowed</i>	100	72.8	<0.01
<i>% Anaemic during Pregnancy</i>	18.8	10.9	NS
<i>% Hypertension with Proteinuria</i>	4.2	12.1	NS
<i>% Complications of Pregnancy</i>	29.2	39.1	NS
<i>% Eclampsia</i>	2.1	0	NS

Early onset of labour and emergency Caesarean section were more common in the Indian women, but otherwise both delivery characteristics were similar (Table 10:2). The duration of gestation was shorter in the Indian women by 5.6 days (unadjusted) as were birth weight and head circumference. The proportion of low birth weight infants tended to be greater in the Indians but this did not achieve statistical significance. Adjustments were made for confounding factors such as maternal age, gestational age and maternal height by analysis of variance (Table 10:3). Between group differences had a larger effect than smoking on birth weight and head circumference but a smaller effect on length.

TABLE 10:2

Delivery and infant characteristics

	Indian vegetarians (n=48)	Controls (n=98)	Statistical Significance
<u><i>Delivery Characteristics</i></u>			
<i>Early onset of labour (%)</i>	10.4	1.1	$p < 0.02$
<i>Spontaneous (%)</i>	77.1	76.1	NS
<i>Forceps (%)</i>	6.3	7.6	NS
<i>Emergency caesarean (%)</i>	10.4	2.2	$p < 0.05$
<i>Elective caesarean (%)</i>	2.1	5.4	NS
<i>Breech (%)</i>	0	4.3	NS
<i>Ventouse (%)</i>	4.2	4.3	NS
<i>Fetal distress</i>	25.0	38.0	NS
<u><i>Infant Characteristics</i></u>			
<i>Jaundice (%)</i>	18.8	23.9	NS
<i>Congenital defects (%)</i>	4.2	4.0	NS
<i>Duration of gestation Mean 95CI</i>	38.7 (38.1-39.3)	39.5 (39.1-39.8)	$p < 0.024$
<i>Birth weight Mean 95CI</i>	3102 (2926-3278)	3449 (3343-3554)	$p < 0.001$
<i>Length Mean 95CI</i>	50.9 (49.9-52.0)	52.0 (51.3-52.7)	NS
<i>Head circumference Mean 95CI</i>	33.6 (33.0-34.2)	34.7 (34.3-35.0)	$p < 0.001$
<i>AGPAR index at 1 minute</i>	8.5 (8.1-8.9)	8.5 (8.2-8.7)	NS
<i>AGPAR index at 5 minutes</i>	9.5 (9.3-9.7)	9.5 (9.4-9.6)	NS
<i>% infants < 2500 g</i>	12.5	3.3	NS

TABLE 10:3

Influence of smoking, male sex and group on birthweight, length and head circumference after adjustment for maternal age, gestational age and maternal height in total sample

	Mean difference	Statistical significance
<u>Birth Weight (g)</u>		
Group (Hindu vegetarian)	-240	$P=0.008$
Male sex	+178	$P=0.013$
Smoking	-158	$P=0.088$
<u>Head Circumference (cm)</u>		
Male sex	0.48	$P<0.001$
Group (Hindu vegetarian)	-0.87	$P=0.001$
Smoking	-0.48	$P=0.078$
<u>Length (cm)</u>		
Male sex	2.22	$P<0.001$
Smoking	-2.1	$P=0.003$
Group (Hindu vegetarian)	-1.32	$P=0.049$

Table 10:4 shows the characteristics of the 32 pairs who were matched for parity, maternal age, sex of the infant and gestational age. Birth weight, head circumference and length remained significantly lower in the Indian infants.

TABLE 10:4

Characteristics of the sample which was matched for sex of infant, maternal age, parity and gestational age

	Indian vegetarians		Caucasian controls		Statistical significance
	Mean	95% CI	Mean	95% CI	
Birth weight* (g)	3179	3043-3315	3482	3346-3618	$p=0.017$
Length* (cm)	51.0	50.1-51.9	53.0	52.1-53.9	$p=0.028$
Head circumference* (cm)	34.0	33.5-34.4	34.7	34.3-35.1	$p=0.028$

* adjusted for sex of infant, maternal age, smoking habits, maternal height and gestational age.

Table 10:5 shows the fatty acid composition of plasma phospholipids and Table 10:6 the concentrations of plasma phospholipid fatty acids in cord blood in the Indians and their gestational age and sex matched controls. Complete analyses were only obtained on 27 pairs. Total plasma phospholipid concentrations were greater in the Indians. The proportions of arachidonic acid (20:4n-6) tended to be higher but did not achieve statistical significance. However, the plasma concentration of phospholipid arachidonic acid was greater. Both the proportion and concentration of docosapentaenoic acid (22:5n-6) were significantly greater and those of DHA (22:6n-3) were significantly lower in the Indians than in the controls. The proportion of 20:3n-9 was low in both groups and the proportion of palmitic acid (16:0) was significantly lower in the Indian group.

TABLE 10:5

Composition of plasma phospholipids (wt%) from cord blood in 27 Indian vegetarians and age-matched Caucasian controls

Fatty Acids	Indian Vegetarians		Controls		Difference		P Value
	Mean	SEM	Mean	SEM	Mean	SEM	
16:0	27.33	0.782	30.03	0.817	-2.69	1.085	0.02
16:1	0.66	0.128	0.81	0.140	-0.148	0.215	0.550
18:0	13.91	0.506	13.06	0.410	0.844	0.669	0.218
18:1	12.01	0.641	12.248	0.395	-0.237	0.649	0.715
18:2	8.01	0.393	7.90	0.504	0.111	0.609	0.857
20:3n-9	0.71	0.102	0.60	0.057	0.111	0.114	0.338
20:3n-6	4.64	0.231	4.84	0.190	-0.20	0.293	0.501
20:4n-6	17.1	0.80	15.71	0.587	1.40	0.981	0.165
20:5n-3	0.32	0.118	0.43	0.044	0.110	0.131	0.398
22:4n-6	1.64	0.115	1.36	0.100	0.28	0.167	0.104
22:5n-6	2.34	0.158	1.58	0.126	0.759	0.172	0.001
22:5n-3	0.45	0.040	0.51	0.038	-0.058	0.049	0.257
22:6n-3	4.01	0.358	5.84	0.305	-1.826	0.503	0.001

TABLE 10:6

Phospholipid fatty acid concentrations in mg/l in cord plasma of 27 Indian vegetarians and age-matched controls

Fatty Acids (mg/l)	IV		Controls		Difference		P Value
	Mean	SE	Mean	SE	Mean	SE	
16:0	218	10.9	205	8.9	13	12.4	0.30
16:1	5.5	1.20	5.8	1.2	0.3	1.75	0.90
18:0	111	6.2	90	4.3	21	6.1	0.002
18:1	100	9.6	86	5.7	14	10.6	0.21
18:2	63	3.8	57	6.2	6	6.6	0.33
20:3n-9	5.9	0.88	4.3	0.49	1.6	1.04	0.127
20:3n-6	37	2.7	34	2.2	3	3.7	0.36
20:4n-6	136	10.0	106	4.8	30	11.0	0.012
20:5n-3	2.7	1.08	3.2	0.47	0.5	0.65	0.66
22:4n-6	13.5	1.45	9.5	0.95	4.0	1.75	0.029
22:5n-6	18.7	1.65	10.8	0.98	7.9	1.79	0.000
22:5n-3	3.7	0.48	3.6	0.28	0.1	0.52	0.887
22:6n-3	32	4.6	40	2.8	8	5.4	0.164
Total	811	45.0	696	34.2	115	50.1	0.030

Table 10:7 shows the fatty acid composition of cord artery phospholipids in 31 pairs of samples. Lower proportions of lauric (12:0), palmitic (16:0), stearic (18:0), oleic (18:1n-9) and DHA (22:6n-3) and higher proportions of eicosatrienoic acid (20:3n-9), dihomogammalinolenic acid (20:3n-6), arachidonic acid (20:4n-6) and docosapentaenoic acid (22:5n-6) were found in the Indian samples compared with their controls. The proportion of 20:3n-9 was high in both Indian and control samples. The elongation product of this fatty acid (22:3n-9) was also tentatively identified. The observed difference between the ratios of 20:3n-9/20:4n-9 was not significant.

TABLE 10:7**Composition (wt%) of cord arterial phospholipid fatty acids**

Fatty Acids	Indian Vegetarians		Caucasian Controls		Difference		P Value
<i>12:0</i>	<i>1.03</i>	<i>0.028</i>	<i>1.18</i>	<i>0.031</i>	<i>-0.15</i>	<i>0.041</i>	<i>0.001</i>
<i>14:0</i>	<i>4.96</i>	<i>0.070</i>	<i>5.14</i>	<i>0.157</i>	<i>-0.177</i>	<i>0.164</i>	<i>0.287</i>
<i>16:0</i>	<i>16.71</i>	<i>0.260</i>	<i>18.48</i>	<i>0.396</i>	<i>-1.774</i>	<i>0.518</i>	<i>0.002</i>
<i>16:1</i>	<i>2.067</i>	<i>0.052</i>	<i>1.95</i>	<i>0.060</i>	<i>0.1194</i>	<i>0.074</i>	<i>0.116</i>
<i>18:0</i>	<i>10.45</i>	<i>0.158</i>	<i>11.21</i>	<i>0.184</i>	<i>-0.76</i>	<i>0.238</i>	<i>0.003</i>
<i>18:1</i>	<i>13.00</i>	<i>0.317</i>	<i>14.47</i>	<i>0.389</i>	<i>-1.474</i>	<i>0.441</i>	<i>0.002</i>
<i>18:2n-6</i>	<i>1.56</i>	<i>0.107</i>	<i>1.52</i>	<i>0.139</i>	<i>0.048</i>	<i>0.174</i>	<i>0.782</i>
<i>20:3n-9</i>	<i>3.74</i>	<i>0.187</i>	<i>3.14</i>	<i>0.182</i>	<i>0.5968</i>	<i>0.248</i>	<i>0.023</i>
<i>20:3n-6</i>	<i>1.76</i>	<i>0.081</i>	<i>1.29</i>	<i>0.089</i>	<i>0.47</i>	<i>0.119</i>	<i>0.000</i>
<i>20:4n-6</i>	<i>13.87</i>	<i>0.354</i>	<i>13.12</i>	<i>0.329</i>	<i>0.748</i>	<i>0.338</i>	<i>0.035</i>
<i>22:3n-9</i>	<i>2.18</i>	<i>0.125</i>	<i>1.72</i>	<i>0.104</i>	<i>0.45</i>	<i>0.167</i>	<i>0.012</i>
<i>22:4n-6</i>	<i>3.40</i>	<i>0.122</i>	<i>3.29</i>	<i>0.106</i>	<i>0.1097</i>	<i>0.159</i>	<i>0.495</i>
<i>22:5n-6</i>	<i>4.15</i>	<i>0.156</i>	<i>3.19</i>	<i>0.150</i>	<i>0.95</i>	<i>0.205</i>	<i>0.000</i>
<i>22:6n-3</i>	<i>4.05</i>	<i>0.173</i>	<i>5.75</i>	<i>0.191</i>	<i>-1.71</i>	<i>0.237</i>	<i>0.000</i>
<i>20:3n-9/ 20:4n-6</i>	<i>0.28</i>	<i>0.019</i>	<i>0.25</i>	<i>0.018</i>	<i>0.03</i>	<i>0.024</i>	<i>0.184</i>
<i>22:5n-6/ 22:6n-3</i>	<i>1.10</i>	<i>0.068</i>	<i>0.58</i>	<i>0.033</i>	<i>0.521</i>	<i>0.069</i>	<i>0.000</i>

10:4 Discussion

The lower birth weights reported in the Indian subjects of this study are in agreement with previous reports and cannot be attributed to the usual predictions such as smoking habits, parity, maternal age, maternal height and gestational age. Although there was a tendency for prematurity and early onset of labour, this alone does not explain the deficit in weight.

In Chapter 6, higher concentrations of arachidonic acid were reported in the plasma phospholipids of Indian vegetarian women and Stammers *et al.* (1989) reported a higher proportion of arachidonic acid in the free fatty acid fractions. Prostaglandin metabolites of arachidonic acid, in particular prostaglandin $E_{2\alpha}$, are involved in parturition. It is possible that the higher proportion of arachidonic acid in the maternal lipids could exaggerate their influence. Lower proportions of DHA were found in both plasma and cord phospholipids in the Indian samples. This would be expected as lower proportions of these fatty acids are found in the plasma phospholipids of Indian vegetarian women. The lower proportion of 22:6n-3 was compensated for by a higher proportion of 22:5n-6. This study is the first to demonstrate differences in the proportions of these fatty acids in cord plasma and arterial phospholipids in man.

It is well-known from animal studies that the proportion of 22:6n-3 and 22:5n-6 can be manipulated by altering the ratio of linoleic/linolenic acid in the diet. Whether the size of the changes reported in this study are sufficient to result in changes in physiological function is uncertain. Animals studies (Tinoco *et al.*, 1978; Neuringer and Connor, 1989) have failed to show any influence of altering the ratio of n-6/n-3 fatty acids on birth weight.

The results of this study confirm the presence of a significant proportion of 20:3n-9 in cord artery phospholipids. The ratio of 20:3n-9/20:4n-6 has traditionally been used as a biochemical index of linoleic acid deficiency. It should be noted that the proportions of metabolites of linoleic acid tended to be greater in the Indian samples than in the controls. The proportion of 20:3n-9 was also greater. The presence of 20:3n-9 is probably an indication of the high capacity of umbilical artery to desaturate and elongate unsaturated fatty acids. It also needs to be recognised that the elongation metabolite of linoleic acid (20:2n-6) could co-chromatograph with 20:3n-9. In order to definitively

identify the 20:3n-9 it would be necessary to first separate the fatty acid methyl esters according to degree of unsaturation by silver nitrate silicic acid chromatography prior to GLC analysis or carry out combined GLC-mass spectroscopic analyses which were both time-consuming and beyond the scope of this study.

It seems almost certain that the lower birth weights in the Indians are due to diet as there are few genetic differences between Indian Hindus and Muslims. While deficiencies of micronutrients may have an important influence in early pregnancy they are not believed to affect birth weight because of the efficiency of the placenta in extracting them from the maternal circulation. Anaemia for example does not affect birth weight and neither does protein intake. Energy intake is believed to be the major factor influencing birth weight. It was found that energy intakes were low in Indian women. Several studies have shown that women with habitually low energy intakes given additional food energy give birth to heavier babies (Lechtig *et al.*, 1975). Glucose is the main fuel used by the infant in the latter part of pregnancy and its availability to the fetus is a major factor determining fetal growth. For example, diabetic mothers give birth to above average weight infants. The practice of "fasting" in Hindu women could lead to decreased availability of glucose to the fetus. Further studies are clearly needed to investigate the underlying mechanisms for the lower birthweights in Hindu women.

10:5 Summary

1. Information on the birthweight, length and head circumferences was obtained on 48 Hindu vegetarian pregnancies and 96 Caucasian omnivore pregnancies.
2. No differences were observed in antenatal complications, but a significant proportion of the Caucasians were smokers. Early onset of labour and emergency caesarian sections were more common in the Indians and the duration of gestation was shorter.
3. Birthweight, head circumferences and length were lower in the Indians, even after adjusting for maternal height, duration of gestation, parity, sex of infant and smoking habits.
4. The proportions of docosahexaenoic acid (22:6n-3) were lower and those of docosapentaenoic acid (22:5n-6) were greater in cord plasma and cord artery phospholipids of the Indians. The proportions of these fatty acids were not statistically related to differences in birth weight, length or head circumference.
5. It is concluded that the low birth weights in the Indians could be due to the lower energy intakes. However, further research is needed.

CHAPTER 11

FINAL DISCUSSION

There is generally a tendency for the Asian groups living in the UK to be identified culturally as a homogenous group, but in reality they are diverse in terms of religion, culture, language and food habits. The term 'Asian' itself is inadequate to describe such a diverse group of migrants. Similarly, 'vegetarian diets' have all been classed together without considering the dietary preferences and habits of the different groups practising vegetarianism. There is also a general belief that vegetarianism is healthy despite the lack of information on the dietary intakes of the various vegetarian groups. The results of the present study clearly indicate that diets and their effects on health vary considerably within the vegetarian groups of different ethnic origins. However, there is one similarity; low frequency of smoking in both the Indian and Caucasian vegetarians. Conversely, both Caucasian omnivores and vegetarians consumed alcohol while the Indians generally abstained.

This study focuses on the effect of vegetarianism on the health of pre-menopausal women of Indian and Caucasian origin. To date this is the only study to use seven-day weighed inventory to assess nutrient intakes in non-pregnant Indian women as opposed to 24-hour recall method used in earlier studies. Calculation of nutrient intakes using the existing food tables was found to be wholly inadequate because of the limited data on nutrient content of ethnic foods. Much effort was taken in actually analyzing ethnic foods for their nutrient composition. Furthermore, it was found that recipes for the same dishes varied widely between households. Therefore, individual recipes were collected and incorporated into their respective nutrient analysis. Unlike the omnivorous diet where fat is intrinsically present in the foods, vegetarian diets both Indian and Caucasian contained added fat which varied with the type of fat being used in the household. Samples of household fats (cooking oils and spreading fats) were also obtained and analysed for their fatty acid composition and the data used to calculate individual fatty acid intakes. The difficulties encountered in accurately assessing nutrient intakes clearly indicate a need for food tables to be extended to include nutrient composition of a wider variety of ethnic foods.

The prevalence of nutritional problems in the Indians, first raises the question of adequacy of their diet and secondly the extent to which they are congruous with the current dietary guidelines. Energy intakes of all the groups were lower than the estimated average requirement (COMA, 1991). However, estimates of energy intakes

from weighed dietary inventories depend on the accuracy with which the subjects record their food intakes.

None of the subjects studied were obese but the proportion of body fat was greater in the Indians and so was the sum of skinfolds. Energy expenditure can be estimated in the subjects by double-labelled water technique which was beyond the scope of this study.

The popular assumption made on vegetarian diets is that they are high in dietary fibre. The dietary fibre intakes of the Indian vegetarians were considerably lower than those of Caucasian vegetarians. In fact, they were similar to the fibre intakes of the omnivores. Yet, the faecal bulk was the greatest in the Indians. Future studies should focus on the effects of non-starch polysaccharides and other fibre fractions in the Indian diets.

The prevalence of nutritional anaemias in pre-menopausal women suggests the intake of micronutrients, particularly iron, vitamin B₁₂ and folate, needs consideration. Iron intakes were similar in all the groups and marginally below the current reference value for iron intake (COMA, 1991). However, low ferritin concentrations found in the vegetarians imply that the availability of non-haem iron needs consideration, particularly in pre-menopausal women as they are in the reproductive phase and are also likely to have menstrual losses of iron. Sources of vitamin B₁₂ in the vegetarian diet are limited and low intakes found in this study indicate a need for augmentation. Vegetarians should be made aware of the wide range of processed foods which are supplemented with vitamin B₁₂. The Indians could be easily encouraged to consume breakfast cereals, as breakfast is the most westernized meal of the day.

Folate status as measured by the concentrations in the erythrocytes was the lowest in the Indians, although the intakes were above the average requirement. However, both vitamin B₁₂ and folate are heat labile and subject to cooking losses. Milk is a fair source of vitamin B₁₂ but the practice of boiling milk to make tea or yoghurt in the Indian households could destroy all the heat sensitive vitamins. Vegetables are a good source of folate but Indian cuisine requires cooking of most vegetables, where folate may be lost. Folate intakes were also lower in the omnivores. The low folate intakes found in the present study lend support to the recent MRC recommendations for folate

supplementation prior to pregnancy (MRC Vitamin Study Group, 1991), and the consequent decrease in neural tube defects in infants. The other haemopoietic nutrient which may be of importance is copper, the intakes of which were significantly lower in the Indian women. The common complaint encountered by general practitioners treating Indian patients is that of weakness, lethargy and difficulty in carrying out household chores after a full working day (Brent, personal communication). Although, these patients have normal haemoglobin levels they may benefit from iron, vitamin B₁₂ and folate supplements. Anaemia was not significantly more prevalent in Hindu vegetarians during pregnancy probably due to supplementation during anti-natal care.

The major macronutrient influencing disease is dietary fat. Much debate has centred on the desirable intakes of fat and the quality of dietary fat. The current guidelines by COMA (1991) for total fat intake are: 33% of the total energy from fat, 10% from saturated fatty acids and 6% from polyunsaturated fatty acids. These figures are somewhat arbitrary. None of the groups in this study meet the recommendations for total fat and saturated fatty acids while polyunsaturated fatty acid intakes were in accordance with the recommendations. A significant relationship between saturated fatty acid intake and plasma cholesterol was found in these subjects. Those subjects with plasma cholesterol levels higher than 5.2 mmol/l may benefit from reduction in saturated fat intake. However, upper body fat distribution was a better predictor of plasma cholesterol. A majority of the Indians had cholesterol levels below 5.2 mmol/l and a normal lipid profile. It is recognized that these subjects were younger (25-40) and menopause may elevate plasma cholesterol in women. Since a higher proportion of the Indian women were above the standard weight for height and also had waist/hip ratios greater than 0.8, weight loss now maybe beneficial in the long term.

The Indians differed from the Caucasians by the presence of two important risk factors; HDL-cholesterol particularly the HDL₂ fraction and Apo(a) concentrations in plasma. Both those factors were associated with centripetal fat distribution. It is already established that low HDL-cholesterol is associated with upper body obesity. Further studies are needed to confirm whether Lp(a) concentrations are related to body fat patterning.

There is evidence that SHBG, is a genetically controlled protein and is instrumental in weight gain and fat patterning. Decreased levels of SHBG are usually accompanied by increasing waist/hip ratio, increasing size of abdominal but not femoral adipocytes, decreasing levels of HDL-cholesterol, increasing plasma glucose and insulin sensitivity. The gamut of observations have all been made in studies on Indians. However, there are no reports of adipocyte measurements. Notable is the apparent centripetal distribution of fat seen in diabetes and its relationship to cardio-vascular risk factors such as gout, hypertension and hyperlipidaemia. Future research needs to be focused on the possibilities of modifying the pattern of fat distribution in the body.

It has been suggested that the ratio of n-6/n-3 fatty acids in the diet may influence risk of CHD and cancer. The ratio was high in the Indians and lower levels of n-3 fatty acids were found in plasma lipids. It is uncertain whether the differences observed are of any patho-physiological significance. It has also been suggested that n-3 fatty acids play an important role in the modulation of eicosanoid formation from arachidonic acid. It might be worthwhile conducting studies on the effects of supplements of n-3 fatty acids on blood pressure and platelet function in the Indians. However, beneficial effects on bleeding time and blood pressure have been only observed with high doses of n-3 fatty acids (3-5g/day).

Low levels of n-3 fatty acids were found in the cord arteries and plasma of infants born to Hindu mothers. It has been suggested that low levels might impair brain or retinal function, but children brought up as life vegans appear to be quite normal despite low levels of n-3 fatty acids. Also, the educational achievement of the Indian children in the UK is found to be above average.

The modification of LDL-cholesterol by the free radicals is also implicated in atherosclerosis and α -tocopherol as an anti-oxidant can influence this modification. The intakes of vitamin E and plasma levels of α -tocopherol were both low in the Indians. The intake was 0.25 mg/g of PUFA, well below the amount of 0.4 mg/g of PUFA suggested. Augmenting vitamin E intakes in the Indians may be beneficial with regard to their risk of CHD.

The mortality statistics show that the risk of breast and colon cancer is lower in the Indians than in the Caucasians. The results of this study did not show any marked difference in hormone concentrations between groups which could explain the lower rates of breast cancer in the Indians. However, there were differences in the faecal bile acid and neutral sterol profiles between the groups. The markers of risk particularly the ratio of lithocholic/deoxycholic acid was lower in the Indians. The proportion of energy from saturated fat was related to the concentrations of secondary bile acids. The sulphur/nitrogen ratio was also related to the degradation of cholesterol in the gut. These observations are consistent with the repeated findings of epidemiological studies showing relationship between consumption of animal foods and colon cancer.

The ratio of lithocholic/deoxycholic acid was significantly related to faecal pH and pulses in the diet were identified as the major predictors of faecal pH. Although the consumption of pulses was similar in the vegetarian groups the type of pulses varied, chickpea, toor and mung dals were popular with the Indians while the Caucasian vegetarians consumed all types of beans. Despite the lower fibre intakes in the Indians they appear to be at low risk from colon cancer and a higher proportion of Indians had detectable levels of primary bile acids in faeces indicating decreased degradation.

Low birth weight is a problem in both Hindus and Muslims. In the present study a difference of approximately 240 g was observed in birth weights of Hindu babies and Caucasian babies which could not be explained by the effects of smoking nor the influence of stature. Low maternal energy intakes influence birth weight. Fasting is observed by most Hindu women which could effect the availability of energy to the growing fetus. However, this remains a speculation as dietary intakes were not recorded by these mothers. As low birth weight is known to influence brain development and neurocognitive abilities in school age children (Hack *et al.*, 1991), future research should focus on the possible causes of low birth-weight in the Indians.

Development, industrialisation and affluence, while enabling the prevention of infectious diseases, has now to cope with the upsurge of CHD and cancer. Health and well-being of an individual or a population is dependent on the interaction of the innate with the environment in which he lives. Migrant populations around the world give us the opportunity to study these interactions. Migrant studies have shown that with

increasing length of stay in the host country, they gradually acquire the disease pattern of that country, implying the importance of environmental influences on health and disease.

Indian immigration to the UK is fairly recent and therefore it is not possible to draw firm conclusions on the effects of the new environment on their health. However, mortality statistics show that they are more at risk from CHD and diabetes than the local population. It is not possible to directly compare the incidence of disease or the causes of mortality in the home country, simply due to the lack of reliable statistics. However, statistics from urban India do show similarities in incidence of 'Western diseases' or the markers of disease between the migrant Indians and those in urban India. Indian migrants in the UK have a more severe form CHD than the local population. Reports of their susceptibility to CHD and diabetes from other countries with substantial numbers of Indian migrants suggest that their susceptibility could be innate or might have been acquired in the new country. Therefore, genetic factors influencing health of the Indians cannot be ignored. Advances in molecular biology may be able to answer these questions in the future. An appreciation of genetic factors may be rewarding in the prevention and therapy of diseases.

Diet is perhaps the most predominant of all environmental factors influencing health. Migration to a new country and acculturation inevitably lead to some changes in individual food choice and diet. Although some acculturation has occurred in the Indians, diet still remains essentially the same, aided by the ready availability of ethnic foods. In fact, acculturation in diet is seen in the opposite direction with the ever increasing popularity of ethnic foods with the local UK population. Religion often dictates food choice. Hinduism advocates vegetarianism as it is associated with 'ahimsa'. A majority of the Hindu migrants continue to be lacto-vegetarians especially women. In the Asian household, women generally are responsible for food preparation for the family and are likely to continue the dietary practices of the home country. Men may indulge in non-vegetarian foods on occasions outside the home. However, it remains to be seen whether these dietary habits will persist in the future generations of ethnic groups born in the UK.

This study revealed that the Indians are generally in good health but some areas need attention. Nutritional knowledge and awareness are associated with health and well-being. There is a need to target nutrition education towards Asians as a whole, especially the women in order to increase their awareness of health issues. There is evidence of low uptake of health services in this ethnic group possibly due to cultural and linguistic barriers. Lack of uptake of preventive measures such as breast screening programmes or cervical cytology is of concern as it may have a lasting effect on long-term health. Although the National Health Service includes a good number of Asian doctors, there are few para-medics of Asian origin such as community nurses, midwives and health visitors who are vital for communication with the women of the household. Non-Asian health workers also need to improve their knowledge and understanding of the cultural features and diversities within the Asian community, to be able to provide the appropriate advice and service.

Another area that needs emphasis is the role of exercise and fitness in improving the health of the Asian community. No studies have actually measured the physical activity levels in the Asians, but there are cultural reasons for some women to be confined to their homes. Exercise in public places may not be acceptable to women and therefore they may need exclusive facilities. There is evidence of improvement in insulin sensitivity with exercise and it could be regarded as an effective measure in tackling obesity and the epidemic of CHD and diabetes in this group.

AREAS FOR FURTHER RESEARCH

1. Since there is a high incidence of Diabetes mellitus in the Indians, further studies should concentrate on the early detection and treatment of any disturbances in glucose and insulin metabolism with a view to avoid other long-term complications of diabetes.
2. Alternative risk factors, other than plasma cholesterol, smoking habits and high blood pressure are needed to explain the high rates of CHD in the Indians.
3. Future research should focus on the genetic aspects of obesity, body fat patterning and the propensity for diabetes and CHD in the Indians.
4. There is a need for research in the area of exercise and its effect on distribution of body fat.
5. The influence of n-3 fatty acids on the risk of CHD in the Indians could be studied by conducting controlled trials.
6. Future research should be focused on the levels of iodine intake, its status in the vegetarians and the effects on energy metabolism.
7. Other reasons for low birthweight in the Hindu vegetarians should be investigated.
8. The relationship between low vitamin B₁₂ status and the high incidence of tuberculosis, suggested recently, requires further research.
9. In-depth studies of the effects of changing lifestyle, diet and acculturation on the health of children born to the ethnic minorities in the UK are needed .
10. More information is required on the nutrient composition of ethnic foods.
11. Toxicological studies on spices used in Indian cuisine could be of value.

REFERENCES

- Abbey, M., Clifton, P., Belling, B. and Nestel, P.J. (1990). Effect of fish oil on lipoproteins, lecithin : cholesterol acyltransferase, and lipid transfer protein activity in humans. *Arteriosclerosis* 10: 85-94.
- Abdulla, M., Anderson, I., Asp, N.G., Berthelsen, K., Birkhed, D., Dencker, I., Johansson, C.G., Jagestad, M., Kolar, K., Nair, B.M., Nilsson-Ehle, P., Norden, A., Rassner, S., Akesson, B. and Ockerman, P.A. (1981). Nutrient intake and health status of vegans. Chemical analysis of diets using the duplicate portion sampling technique. *American Journal of Clinical Nutrition* 34: 2464-2477.
- Abraham, R., Campbell-Brown, M., Haines, A.P., North, W.R.S., Hainsworth, V. and McFayden, I.R. (1985). Diet during pregnancy in an Asian community in Britain - energy, protein, zinc, copper, fibre and calcium. *Human Nutrition : Applied Nutrition* 39A: 23-35.
- Adelstein, A.M. and Marmot, M.G. (1989). The health of migrants in England and Wales: causes of death. In: *Ethnic Factors in Health and Disease* (eds Cruikshank, J.K. and Beever, D.G.). London: Wright.
- Adlercreutz, H., Hockerstedt, K., Bannwart, C., Bloign, S., Hamalainen, E., Fotsis, T. and Ollus, H. (1987). Effect of dietary components, including lignans and phyto-oestrogens, on enterohepatic circulation and liver metabolism of oestrogens and on SHBG. *Journal of Steroid Biochemistry* 27: 1135-1144.
- Alibhai, Y. (1989). In: *The Observer Magazine*, 19 November.
- Ames, B.N. (1979). Identifying environmental chemicals causing mutation and cancer. *Science* 204: 587-594.
- Anderson, B.M., Gibson, R.S. and Sabry, J.H. (1981). The iron and zinc status of long-term vegetarian women. *American Journal of Clinical Nutrition* 34: 1042-1049.
- Anderson, D.W., Nichol, A.V., Pau, S.S. and Lindgren, F.T. (1978). High density lipoprotein distribution. Resolution and determination of three components in a normal population sample. *Atherosclerosis* 29: 161-179.
- Angelico, F., Amodeo, P., Guccione, P., Montali, A., Menotti, A., Ricci, G. and Urbinatti, G.C. (1982). Dietary red blood cell fatty acid changes after a four year dietary intervention in the Rome Project of Coronary Heart Disease Prevention. *Clinica e Terapia Cardiovascolare* 1: 1-7.
- Anwar, M. (1981). In: *Between Two Cultures: A study of relationships in generations in the Asian Community in Britain*. London: Commission for Racial Equality.
- Arborgh, B., Eklund, A., Norman, A. and Strandvik, B. (1980). Urinary bile acid excretion in correlation to liver histopathology in cystic fibrosis. *Scandinavian Journal of Gastroenterology* 15: 73-80.

- Aries, V.C., Crowther, J.S., Draser, B.S. and Hill, M.J. (1969). Degradation of bile salts by human intestinal bacteria. *Gut* 10: 575-577.
- Aries, V.C. and Hill, M.J. (1970). Degradation of steroids by intestinal bacteria 1: Deconjugation of bile salts. *Biochimica et Biophysica Acta* 202: 526.
- Aries, V.C., Crowther, J.S., Drasar, B.S., Hill, M.J. and Ellis, F.R. (1971). The effect of a strict vegetarian diet on the faecal flora and faecal steroid concentration. *Journal of Pathology* 103: 54-56.
- Armstrong, B. and Doll, R. (1975). Environmental factors and cancer incidence in different countries with special reference to dietary practices. *International Journal of Cancer* 15: 617-631.
- Armstrong, B.K., Clarke, H., Martin, C., Ward, W., Norman, N. and Maserei, T. (1979). Urinary sodium and blood pressure in vegetarians. *American Journal of Clinical Nutrition* 33: 2472-2476.
- Armstrong, B.K., Davis, R.E., Nicol, D.J., Van Merwick, A.J. and Larwood, C.J. (1974). Haematological vitamin B₁₂ and folate studies on Seventh-Day Adventist vegetarians. *American Journal of Clinical Nutrition* 27: 712-718.
- Ashwell, M., Chinn, S., Stalley, S. and Garrow, J.S. (1982). Female fat distribution - a simple classification based on two circumference measurements. *International Journal of Obesity* 6: 143-152.
- Aurora, G.S. (1967). *The New Frontiersmen*. Bombay: Popular Prakashan.
- Avons, P. (1985). Marqueurs de l'apport alimentaire en acides gras: leur utilisation dans les enquêtes épidémiologiques. *Revue d'Epidémiologie et de Santé Publique* 33: 312-323.
- Aylsworth, C.F., Van Vugt, D.A., Sylvester, P.W. and Meites, J. (1984). Failure of high dietary fat to influence serum prolactin levels during the oestrous cycle in female Sprague-Dawley rats. *Proceedings of the Society for Experimental Biology and Medicine* 175: 25-29.
- Bachorik, P.S., Walker, R.E. and Virgil, D.G. (1984). High density lipoprotein cholesterol in heparin-manganese supernates determined with the Dow enzyme method after precipitation with HCO₃. *Clinical Chemistry* 30: 839-842.
- Badwe, R.A., Gregory, W.M., Chaudary, M.A., Richards, M.A., Bentley, A.E., Rubens, R.D. and Fentman, I.S. (1991). Timing of surgery during menstrual cycle and survival of premenopausal women with operable breast cancer. *The Lancet* 337: 1261-1264.
- Baker, S.J. and De Maeyer, E.M. (1979). Nutritional anaemia: its understanding and control with special reference to the work of WHO. *American Journal of Clinical Nutrition* 32: 368-417.

- Balarajan, R. and Yuen, P. (1986). British smoking and drinking habits: variation by country of birth. *Community Medicine* 8: 237-239.
- Balarajan, R., Bulusu, L., Adelstein, A.M. and Shukla, V. (1984). Patterns of mortality among migrants to England and Wales from the Indian subcontinent. *British Medical Journal* 289: 1185-1187.
- Balarajan, R., Soni Raleigh, V. and Botting, B. (1989). Sudden infant death syndrome and post-neonatal mortality in immigrants in England and Wales. *British Medical Journal* 298: 717-720.
- Balch, C.M., Doughartz, P.A. and Tilden, A.B. (1982). Excessive prostaglandin E₂ production by suppressor monocytes in head and neck cancer patients. *Annals of Surgery* 196: 645-650.
- Bang, H.O. and Dyerberg, J. (1980). Lipid metabolism in Greenland Eskimos. *Advances in Nutrition Research* 3: 1-40.
- Barbosa, J.C., Schultz, T.D., Filley, S.J. and Nieman, D.C. (1990). The relationship among adiposity, diet, and hormone concentrations in vegetarian and non-vegetarian post-menopausal women. *American Journal of Clinical Nutrition* 51: 798-803.
- Baumgartner, R.N., Roche, A.F., Chumlea, W.C., Siervogel, R.M. and Glueck, C.J. (1987). Fatness and fat patterns: associations with plasma lipids and blood pressure in adults, 18 to 57 years of age. *American Journal of Epidemiology* 126: 614-628.
- Beevers, D.G. and Cruikshank, J.K. (1981). Age, sex, ethnic origin and hospital admission for heart attack and stroke. *Postgraduate Medical Journal* 5: 763-765.
- Behrens, W.A., Thompson, J.N. and Madère, R. (1982). Distribution of α -tocopherol in human plasma lipoproteins. *American Journal of Clinical Nutrition* 35: 691-696.
- Belanger, A., Locong, A., Noel, C., Cusan, L., Dupont, A., Prevost, J., Caron, S. and Seigny, J. (1989). Influence of diet on plasma steroid and sex hormone binding globulin in adult men. *Journal of Steroid Biochemistry* 32: 829-833.
- Bennion, L.J., Drobny, E., Knowler, W.C., Ginsberg, R.L., Garnick, M.B., Adler, R.D. and Duane, W.C. (1978). Sex differences in the size of bile acid pools. *Metabolism* 27: 961-969.
- Berlin, E., Matusik, E.J. and Young, C. (1980). Effect of dietary fat on the fluidity of platelet membranes. *Lipids* 15: 604-608.
- Berrino, F. and Muti, P. (1989). Mediterranean diet and cancer. *European Journal of Clinical Nutrition* 43(Suppl.2): 49-55.

- Berrino, F., Muti, P. and Pisani, P. (1988). Overview of the etiological hypothesis linking endogenous steroid hormones and breast cancer. In: *Diet, Hormones and Cancer: Methodological Issues for Prospective Studies*, IARC Technical Series No.4. Lyon: WHO.
- Beynen, A.C. and Katan, M.B. (1985). Rapid sampling and long term storage of subcutaneous adipose tissue biopsies for determination of fatty acid composition. *American Journal of Clinical Nutrition* 42: 317-322.
- Beynen, A.C. and Katan, M.B. (1989). Impact of dietary cholesterol and fatty acids on serum lipids and lipoproteins in man. In: *The Role of Fats in Human Nutrition* (eds Vergoesen, A.J. and Crawford, M.). London: Academic Press.
- Beynen, A.C., Hermus, R.J.J. and Hautvast, J.G.A.J. (1980). A mathematical relationship between the fatty acid composition of the diet and that of the adipose tissue in man. *American Journal of Clinical Nutrition* 33: 81-85.
- Bieri, J.B., Tolliver, T.J. and Catignani, G.L. (1979). Simultaneous determination of α -tocopherol and retinol in plasma or red cells by high pressure liquid chromatography. *American Journal of Clinical Nutrition* 32: 2143-2149.
- Bindra, G.S. and Gibson, R.S. (1986). Fe status of predominantly lacto-ovo-vegetarian East Indian immigrants to Canada: a model approach. *American Journal of Clinical Nutrition* 44: 643-652.
- Bingham, S., Williams, D.R.R., Cole, T.J. and James, W.P.T. (1979). Dietary fibre and regional large bowel cancer mortality in Britain. *British Journal of Cancer* 40: 456-463.
- Bingham, S.A. (1990). Mechanisms and experimental and epidemiological evidence relating dietary fibre (non-starch polysaccharides) and starch to protection against large bowel cancer. *Proceedings of the Nutrition Society* 49: 153-171.
- Bingham, S.A., Williams, D.R.R. and Cummings, J.H. (1985). Dietary fibre consumption in Britain; new estimates and their relation to large bowel cancer mortality. *British Journal of Cancer* 52: 399-402.
- Bishop, D.T., Meikle, A.W., Slattery, M.L., Stringham, J.D., Ford, M.H. and West, D.W. (1988). The effect of nutritional factors on sex hormone levels in male twins. *Genetic Epidemiology* 5: 43-59.
- Bissenden, J.G., Scott, P.H., King, J., Hallum, H., Mansfield, H.N. and Wharton, B.A. (1981). Anthropometric and biochemical changes during pregnancy in Asian and European mothers having light for gestational age babies. *British Journal of Obstetrics and Gynaecology* 88: 999-1008.
- Black, J. (1985). Paediatrics among ethnic minorities. Asian Families II: a condition that may be found in the children. *British Medical Journal* 290: 830-833.

- Boissonneault, G.A., Elson, C.E. and Pariza, M.W. (1986). Net energy effects of dietary fats on chemically induced mammary carcinogens in F344 rats. *Journal of the National Cancer Institute* 76: 335-338.
- Bonanome, A. and Grundy, S.M. (1988). Effects of dietary stearic acid on plasma cholesterol and lipoprotein levels. *New England Journal of Medicine* 318: 1244-1248.
- Boreh-Johnsen, K., Joner, G. and Mandrup Poulsen, T. (1984). Relation between breast feeding and incidence rates of insulin dependent diabetes mellitus. *The Lancet* ii: 1083-1086.
- Bowker, J. (1983). In: *Worlds of Faith*. London: BBC/Ariel Books.
- Brenner, R.R. (1982). Nutritional and hormonal factors influencing desaturation of essential fatty acids. *Progress in Lipid Research* 20: 41-47.
- Britt, R.P., Harper, C. and Spray, G.H. (1971). Megaloblastic anaemia among Indians in Britain. *Quarterly Journal of Medicine* 40: 499-520.
- Britt, R.P., Hollis, Y. and Keil, J.E. (1983). Anaemia in Asians in London. *Post-graduate Medical Journal* 59: 645-657.
- Brown, C. (1984). *Black and White Britain: The Third PSI Survey*. London: Heinemann.
- Brussard, J.H., Katan, M.B. and Hautvast, J.G.A.J. (1983). Fecal excretion of bile acids and neutral steroids on diets differing in type and amount of dietary fat in young healthy persons. *European Journal of Clinical Investigation* 13: 115.
- Bull, A.W., Soullier, B.K., Wilson, P.S., Haydon, M.T. and Nigro, N.D. (1979). Promotion of azoxy-methane-induced intestinal cancer by high-fat diets in rats. *Cancer Research* 39: 4956.
- Bull, N.L. and Barber, S.A. (1984). Food and nutrient intakes of vegetarians in Britain. *Human Nutrition : Applied Nutrition* 38A: 288-293.
- Burkitt, D.P. (1969). Related disease - related cause. *Lancet* ii: 1229-1231.
- Burkitt, D.P. (1975). Disease and environment: significance of relationships. In: *Refined Carbohydrate Foods and Disease* (eds Burkitt, D.P. and Trowell, H.C.). London: Academic Press.
- Burr, M.L. and Butland, B.K. (1988). Heart disease in British vegetarians. *American Journal of Clinical Nutrition* 48: 830-832.
- Burr, M.L. and Sweetman, P.M. (1982). Vegetarianism, dietary fibre and mortality. *American Journal of Clinical Nutrition* 36: 873-876.

- Burr, M.L., Bates, C.J., Fehily, A.M. and St Leger, A.S. (1981). Plasma cholesterol and blood pressure in vegetarians. *Journal of Human Nutrition* 35: 437-441.
- Burr, M.L., Fehily, A.M., Gilbert, J.F., Rogers, S., Holliday, R.M., Sweetnam, P.M., Elwood, P.C. and Deadman, N.M. (1989). Effects of change in fat, fish and fibre intakes on death and myocardial infarction: Death and Reinfarction Trial (DART). *The Lancet* ii: 757-760.
- Burslem, J., Schonfeld, G., Howald, M.A., Weidman, S.W. and Miller, J.P. (1978). Plasma apoprotein and lipoprotein lipid levels in vegetarians. *Metabolism* 27: 711-719.
- Burton, G.W. and Ingold, K.U. (1984). β -carotene: an unusual type of lipid antioxidant. *Science* 224: 569-573.
- Byers, T. (1988). Diet and cancer. Any progress in the interim? *Cancer* 62: 1713-1724.
- Callmer, E. (1987). Description of the dietary methods evaluated in the Malmö methodological study. In: *Prospective Studies on Diet and Cancer* (Riboli, E. and Saracci, R., eds), IARC Technical Report series. Lyon: IARC.
- Campbell, M., Lofters, W.S. and Gibbs, W.N. (1982). Rastafarianism and the vegan syndrome. *British Medical Journal* 285: 1617-1618.
- Campbell-Brown, M., Ward, R.J., Haines, A.P., North, W.R.S., Abraham, R. and McFadyen, I.R. (1985). Zinc and copper in Asian pregnancies - Is there evidence for nutritional deficiency? *British Journal of Obstetrics and Gynaecology* 92: 875-885.
- Carlson, E., Kipps, M., Lockie, A. and Thompson, J. (1985). A comparative evaluation of vegan, vegetarian and omnivore diets. *Journal of Plant Foods* 6: 89-100.
- Carlson, E., Knipps, M. and Thompson, J. (1984). Influences on the food habits of some ethnic minorities in the U.K. *Human Nutrition : Applied Nutrition* 38A: 85-98.
- Carr-Hill, R. and Chadha-Boreham, H. (1988). In: *Britain's Black Population: A New Perspective*, 2nd edition "The Radical Statistics Race Group". Aldershot: Gower Publishing Co. Ltd.
- Carroll, K.K. (1986). Experimental studies on dietary fat and cancer in relationship to epidemiological data. In: *Dietary Fat and Cancer Progress in Clinical and Biological Research* Vol.222. New York: Alan R. Liss Inc., pp 231-248.
- Carroll, K.K. and Khor, H.T. (1971). Effects of level and type of dietary fat on incidence of mammary tumors induced in female Sprague-Dawley rats by 7,12-dimethylbenz(α)anthracene. *Lipids* 6: 415-420.

- Carroll, K.K. and Khor, H.T. (1975). Dietary fat in relation to tumorigenesis. *Progress in Biochemistry and Pharmacology* 10: 308-353.
- Central Statistical Office (1986). *Social Trends 16*. London: HMSO.
- Central Statistical Office (1989). *Social Trends 19*. London: HMSO.
- Central Statistical Office (1990). *Social Trends 20*. London: HMSO.
- Chan, P., Didato, F. and Cohen, C.A. (1975). High dietary fat, elevation of rat serum prolactin and mammary cancer. *Proceedings of the Society of Experimental Biology and Medicine* 149: 133-135.
- Chanarin, I., Malkowska, V., O'Hara, A.M., Rinsler, M.G. and Price, A.B. (1985). Megaloblastic anaemia in a vegetarian Hindu community. *Lancet* ii: 1168-1172.
- Chanarin, I. and Stephenson, E. (1988). Vegetarian diet and cobalamin deficiency: their association with tuberculosis. *Journal of Clinical Pathology* 41: 759-762.
- Cheah, J.S., Lui, K.F., Yeo, P.P.B., Tam, B.Y., Tan, Y.T. and Ng, Y.K. (1979). Diabetes mellitus in Singapore: results of a countrywide population survey. In: *Epidemiology of Diabetes in Developing Countries* (Ahuja, M.M.S., ed.). New Delhi: Interprint, pp 93-102.
- Cheug, M.C. and Albers, J.J. (1977). The measurement of apolipoprotein AI and AII levels in men and women by immunoassay. *Journal of Clinical Investigation* 60: 43-50.
- Chilvers, C., Fraser, P. and Beral, V. (1979). Alcohol and oesophageal cancer: an assessment of the evidence from routinely collected data. *Journal of Epidemiology and Community Health* 33: 127-133.
- Christie, W.W. (1973). *Lipid Analysis*. Oxford: Pergamon Press.
- Clarson, C.L., Barker, M.J., Marshall, M. and Wharton, B.A. (1982). Secular change in birthweight of Asian babies born in Birmingham. *Archives of Diseases of Childhood* 57: 867-871.
- Cohen, L.A., Thompson, D.D., Maeura, Y. and Weisburger, J.H. (1984). Influence of dietary medium chained triglycerides on the development of N-methylnitrosurea-induced rat mammary tumour. *Cancer Research* 44: 5203-5208.
- Commission for Racial Equality (1985). *Fact Paper 3: Background of Asian Minority Groups*. London: CRE.
- Committee on Medical Aspects of Food Policy (1984). *Diet and Cardiovascular Disease*. Report of the Panel on Diet in Relation to Cardiovascular Disease. DHSS Report on Health and Social Subjects, No.28. London: HMSO.

- Committee on Medical Aspects of Food Policy (1991). *Dietary Reference Values for Food Energy and Nutrients for the United Kingdom*. Report on Health and Social Subjects No.41. London: H.M.S.O.
- Connor, W.E., Witiak, D.T., Stone, D.B. and Armstrong, M.L. (1969). Cholesterol balance and fecal neutral steroid and bile acid excretion in normal men fed dietary fats of different fatty acid composition. *Journal of Clinical Investigation* 48: 1363.
- Conrad, M.E. and Barton, J.C. (1981). Factors affecting iron balance. *American Journal of Haematology* 10: 199.
- Cook, J.D. and Skikne, B.S. (1982). Serum ferritin: a possible model for the assessment of nutrient stores. *American Journal of Clinical Nutrition* 35: 1180-1185.
- Coronary Prevention Group (1986). *Coronary Heart Disease and Asians in Britain*. London: Confederation of Indian Organisations.
- Correa, P. (1981). Nutrition and cancer: epidemiologic correlations. In: *The Practice of Cancer Prevention in Clinical Medicine* (Newell, G.R., ed.). New York: Raven Press.
- Cortese, C., Levy, Y. and Janus, E.D. (1983). Modes of action of lipid-lowering diets in man: studies of apolipoprotein B kinetics in relation to fat. *European Journal of Clinical Investigation* 13: 79-85.
- Crapo, P.A. (1985). Simple versus complex carbohydrate use in diabetic diet. *Annual Review of Nutrition* 5: 95-114.
- Crawford, M.A. and Sinclair, A.J. (1972). Nutritional influences in the evolution of the mammalian brain. In: *Lipids, Malnutrition and the Developing Brain*. Amsterdam: Elsevier.
- Crawford, M.A. and Sinclair, A.J. (1972). Nutritional influences in the evolution of the mammalian brain. In: *Lipids, Malnutrition and the Developing Brain*. Amsterdam: Elsevier.
- Crawford, M.A., Doyle, W., Drury, P., Lennon, A., Costeloe, K. and Leighfield, M. (1989). n-6 and n-3 fatty acids during early human development. *Journal of Internal Medicine* 225(Suppl.1): 159-169.
- Crawford, M.A., Hassam, A.G., Williams, G. and Whitehouse, W.E. (1976). Essential fatty acids and fetal brain growth. *Lancet* i: 452-453.
- Crawford, M.A., Hassam, A.G., Williams, G. and Whitehouse, W.E. (1976). Essential fatty acids and fetal brain growth. *Lancet* i: 452-453.
- Crawford, M.E., Doyle, W., Drury, P., Lennon, A., Costeloe, K. and Leighfield, M. (1989). n-6 and n-3 fatty acids during early human development. *Journal of Internal Medicine* 225(Suppl.1): 159-169.

- Crawley, H. (1988). *Food Portion Sizes. Ministry of Agriculture, Fisheries and Food.* London: HMSO.
- Crowther, J.S., Drasar, B.S., Goddard, P., Hill, M.J. and Johnson, K. (1973). The effect of a chemically defined diet on the fecal flora and fecal steroid concentration. *Gut* 14: 790.
- Cruikshank, J.K. (1989). Cardiovascular disease in black and Indian origin populations outside the USA. In: *Ethnic Factors in Health and Disease* (Cruikshank, J.K. and Beevers, D.G., eds). London: Wright.
- Cruikshank, J.K. and Beevers, D.G. (1989). Migration, ethnicity, health and disease. In: *Ethnic Factors in Health and Disease* (Cruikshank, J.K. and Beevers, D.G., eds). London: Wright.
- Cruikshank, J.K., Jackson, S.H.D., Beevers, D.G., Bannan, L.T., Beevers, M. and Stewart, V.L. (1985). Similarity of blood pressure of blacks, whites and Asians in England. *Journal of Hypertension* 3: 365-371.
- Cummings, J.H., Hill, M.J., Bone, E.S., Branch, W.J. and Jenkins, D.J.A. (1979). The effect of meat protein and dietary fibre on colonic function and metabolism. II. Bacterial metabolites in feces and urine. *American Journal of Clinical Nutrition* 32: 2094-2101.
- Cummings, J.H., Hill, M.J., Jenkins, D.J.A., Pearson, J.R. and Wiggins, H.S. (1976). Changes in fecal composition and colonic function due to cereal fibre. *American Journal of Clinical Nutrition* 29: 1468-1473.
- Cummings, J.H., Wiggins, H.S., Jenkins, D.J.A., Houston, H., Jivraj, T., Drasar, B.S. and Hill, M.J. (1978). Influence of diets high and low in animal fat on bowel habit, gastrointestinal transit time, fecal microflora, bile acid and fat excretion. *Journal of Clinical Investigation* 61: 953.
- Dales, L.G., Friedman, G.D., Ury, H.K., Grossman, S. and Williams, S.R. (1979). A case control study of relationships of diet and other traits to colorectal cancer in American blacks. *American Journal of Epidemiology* 109: 132-144.
- Danaraj, T.J., Acker, M.S. and Danaraj, W. (1959). Ethnic group differences in coronary heart disease in Singapore: an analysis of necroscopy records. *American Heart Journal* 58: 516-526.
- Davies, A.G. and Wheeler, E. (1989). Analysis of the weights of infants of Bangladeshi origin attending two clinics in Tower Hamlets. *Child Care, Health and Development* 15(3): 167-174.
- Davies, J. (1984). In: *Asian Housing in Britain.* Social Affairs Unit.
- Davis, I.R., Marten, R.H. and Sakarny, I. (1960). Iron deficiency anaemia in European and West Indian infants in London. *British Medical Journal* 2: 1426-1428.

- Dayton, S., Pearce, M.L., Goldman, H., Harnish, A., Plotkin, D., Schickman, M., Winfield, M., Zager, A. and Dixon, W. (1968). Controlled trial of a diet high in unsaturated fat for prevention of atherosclerotic complications. *Lancet* ii: 1060-1062.
- de Waard, F. (1975). Breast cancer incidence with particular reference to body weight and height. *Cancer Research* 35: 3351-3356.
- Deakin, N. (1970). *Colour, Citizenship and British Society*. London: Panther Books.
- Dean, J.A. (1960). *Flame Photometry*. London: McGraw Hill.
- Department of Health and Social Security (1982). *Report on Confidential Enquiries into Maternal Deaths in England and Wales*. Reports on Health and Social Subjects No.26. London: HMSO.
- Department of Health and Social Security (1980). *Rickets and Osteomalacia*. Reports on Health and Social Subjects No.19. London: HMSO.
- Department of Health and Social Security (1979). *Recommended Daily Amounts of Food Energy and Nutrients for Groups of People in the U.K.* Reports on Health and Social Subjects No.15. London: HMSO.
- Desprès, J.P. (1991). Obesity and lipid metabolism: relevance of body fat distribution. *Current Opinion in Lipidology* 2(1): 5-15.
- Desprès, J.P., Moojani, S., Lupien, P.J., Angelo, T., Nadeau, A. and Bouchard, C. (1990). Regional distribution of body fat, plasma lipoproteins and cardiovascular disease. *Arteriosclerosis* 10(4): 497-511.
- Doll, R. (1987). Major epidemics of the 20th century from coronary thrombosis to AIDS. *Journal of the Royal Statistical Society* 150: 373-395.
- Doll, R. and Peto, R. (1981). The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. *Journal of the National Cancer Institute* 66: 1191-1308.
- Donaldson, L.J. and Clayton, D.G. (1984). Occurrence of cancer in Asians and non-Asians. *Journal of Epidemiology and Community Health* 38: 203-207.
- Donaldson, L.J. and Taylor, J.B. (1983). Patterns of Asian and non-Asian morbidity in hospitals. *British Medical Journal* 286: 949-951.
- Donegan, W.L., Hartz, A.J. and Rim, A.A. (1978). The association of body weight with recurrent cancer of the breast. *Cancer* 41: 1590-1594.
- Donovan, J. (1977). *We Don't Buy Sickness, it just comes*. Aldershot: Gower Publishing Company.
- Drasar, B.S. and Hill, M.J. (1974). *Human Intestinal Flora*. London: Academic Press.

- Draser, B.S. and Irving, D. (1973). Environmental factors and cancer of colon and breast. *British Journal of Cancer* 27: 167-172.
- Driskell, W.J., Neese, W., Bryant, C. and Bashor, M.M. (1982). Measurement of vitamin A and vitamin E in human serum by high performance liquid chromatography. *Journal of Chromatography* 23: 439-444.
- Duane, W.C. and Hanson, K.C. (1978). Role of gall bladder emptying and small bowel transit in regulation of bile acid pool size in man. *Journal of Laboratory and Clinical Medicine* 92: 858-872.
- Dunn, J.E. (1977). Breast cancer among American Japanese in San Francisco Bay area. *NCI Monographs* 47: 157-160.
- Dunnigan, M.G., McIntosh, W.B., Ford, J.A. and Robertson, I. (1982). Acquired disorders of vitamin D metabolism. In: *Clinical Endocrinology 2 - Calcium disorders* (Heath, D.A. and Marx, S.J., eds). London: Butterworth.
- Dunnigan, M.G., Paton, J.P.J., Haage, S. *et al.* (1962). Late rickets and osteomalacia in the Pakistani community in Glasgow. *Scottish Medical Journal* 7: 159-167.
- Durnin, J.V.G.A. and Rahaman, M.M. (1967). The assessment of the amount of fat in the human body from measurements of skinfold thickness. *British Journal of Nutrition* 21: 681.
- Durnin, J.V.G.A. and Womersley, J. (1974). Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 48 men and women aged from 16-72 years. *British Journal of Nutrition* 32: 77-97.
- Durrington, P.N., Hunt, L., Ishola, M., Arrol, S. and Bhatnagar, D. (1988). Apolipoproteina (a), AI and B and parental history in men with early onset ischaemic heart disease. *Lancet* i: 1070-1073.
- Dwyer, J.T. (1988). Health aspects of vegetarian diets. *American Journal of Clinical Nutrition* 48(3): Supplement, 712-738.
- Dwyer, J.T., Dietz, W.H., Hass, G.H. and Suskind, R.M. (1979). Risk of nutritional rickets among vegetarian children. *American Journal of Diseases of Childhood* 133: 134-140.
- Early Breast Cancer Trialists' Collaboration Group (1988). Effect of adjuvant tamoxifen and of cytotoxic therapy on mortality in early breast cancer. *New England Journal of Medicine* 319(26): 1682-1692.
- Eaton, P.M., Wharton, P.A. and Wharton, B.A. (1984). Nutrient intake of pregnant Asian women at Sorrento Maternity Hospital, Birmingham. *British Journal of Nutrition* 52: 457-468.
- Ehrhardt, P. (1986). Iron deficiency in young Bradford children in different ethnic groups. *British Medical Journal* 292: 90-93.

- Ehrich, M., Ashell, J.E., Van Tassel, R.L., Wilkins, T.D., Walker, A.R.P. and Richardson, N.J. (1979). Mutagens in the feces of 3 South African populations at different levels of risk for colon cancer. *Mutation Research* 64: 231.
- Ellis, F.R. and Mumford, P. (1967). The nutritional status of vegans and vegetarians. *Proceedings of the Nutrition Society* 26: 205-212.
- Ellis, F.R., Holesh, S. and Ellis, J.W. (1975). Incidence of osteoporosis in vegetarians and omnivores. *American Journal of Clinical Nutrition* 25: 555-558.
- Emerit, I., Levy, A. and Cerutti, L. (1983). Suppression of tumour promoter phorbomyristate acetate-induced chromosome breakage by antioxidants and inhibitors of arachidonic acid metabolism. *Mutation Research* 110: 327-335.
- Englyst, H.N., Bingham, S.A., Runswick, S.A., Collinson, E. and Cummings, J.H. (1989). Dietary fibre (NSP) in cereals and cereal products. *Journal of Human Nutrition and Dietetics* 2: 253-271.
- Enstrom, J.E. (1975). Colo-rectal cancer and consumption of beef and fat. *British Journal of Cancer* 32: 432-439.
- Enstrom, J.E. (1978). Cancer and total mortality among active Mormons. *Cancer* 42: 1943-1951.
- Esterbauer, H., Dieber-Rotheneder, M., Striegl, G. and Waeg, G. (1991). Role of vitamin E in preventing the oxidation of low density lipoprotein. *American Journal of Clinical Nutrition* 53: 3145-3215.
- European Atherosclerosis Society (1987). Strategies for the prevention of coronary heart disease: a policy statement of the European Atherosclerosis Society. *European Heart Journal* 8: 77-78.
- Fadden, K., Owen, R. and Hill, M.J. (1984). Steroid degradation along the gastrointestinal tract: the use of the cannulated pig as a model system. *Transactions of the Biochemical Society* 12: 1105-1106.
- Farquhar, J.W. and Ahrens, E.H. (1963). Effects of dietary fats on human erythrocyte fatty acid pattern. *Journal of Clinical Investigation* 42(1): 675.
- Fernandez, F., Kennedy, H. and Todd, E. (1984). Diet and steroid composition in ileostomy fluid. *Transactions of the Biochemical Society* 12: 1103-1104.
- Fernandez, F., Kennedy, H., Hill, M.J. and Truelove, S. (1985). The effect of diet on the bacterial flora of ileostomy fluid. *Microbiologie et Aliments, Nutrition* 3: 47-52.
- Fishman, J. and Martucci, C. (1980). Biological properties of 16 α -hydroxyoestrone: implications in oestrogen physiology and pathophysiology. *Journal of Clinical Endocrinology and Metabolism* 51: 611-615.

- Food and Agricultural Organization (FAO) (1988). *Requirements of Vitamin A, Iron, Folate and Vitamin B₁₂*. Report of a Joint FAO/WHO Expert Consultation. FAO Food and Nutrition Series No.23. Rome: FAO.
- Ford, J., Colhoun, E.M., McIntosh, W.B. and Dunnigan, H.G. (1972). Biochemical response of late rickets and osteomalacia to a chapati-free diet. *British Medical Journal* 3: 446.
- Ford, J.A., McIntosh, W.B., Butterfield, R., Preece, M.A., Pietrek, J., Arrowsmith, W.A., Arthurton, M.W., Turner, W., O'Riordan, J.L.H. and Dunnigan, M.G. (1976). Clinical and sub-clinical vitamin D deficiency in Bradford children. *Archives of Disease in Childhood* 51: 939-943.
- Fosbrooke, A.S. and Wharton, B.A. (1973). Plasma lipids in umbilical cord blood from infants of normal and low birthweight. *Biology of the Neonate* 23: 330.
- Freeland-Graves, J.H., Bodzy, P.W., Ebargit, M.L. and Epwright, M.A. (1980). Zinc status in the vegetarian. *Journal of the American Dietitians Association* 77: 655-661.
- Friedwald, W.T., Levy, R.I. and Fredrickson, S. (1972). Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry* 18: 499-502.
- Frimpong, N.A. and Lapp, J.A. (1989). Effects of moderate alcohol intake in fixed or variable amounts on concentration of serum lipids and liver enzymes in healthy young men. *American Journal of Clinical Nutrition* 50: 987-991.
- Frisch, R.E. (1984). Amenorrhea, vegetarianism and/or low fat. *Lancet* i: 1024-1025.
- Ganapathy, S. and Dhanda, S. (1980). Protein and iron nutrition in lacto-ovo vegetarian Indo-Aryan United States residents. *Indian Journal of Nutrition and Dietetics* 17: 45-52.
- Gandhi, B.M. (1982). Lipoprotein composition of normal healthy subjects in Northern India. *Indian Journal of Medical Research* 75: 393-401.
- Garland, C., Shekelle, R.B., Barrett-Connor, E., Criqui, M.H., Rossof, A.H. and Paul, O. (1985). Dietary Vit D and calcium risk of colorectal cancer: a 19 year prospective study in men. *Lancet* i: 307-309.
- Gear, J.S., Ware, A.C., Fursdon, P.A., Nolan, D.J., Mann, D.I., Broadribb, A.J.M. and Vessey, M.P. (1979). Symptomless diverticular disease and intake of dietary fibre. *Lancet* i: 511-514.
- Gear, J.S.S., Mann, J.I., Thorogood, M., Carter, R. and Jeffs, R. (1980). Biochemical and haematological variables in vegetarians. *British Medical Journal* 280: 1415.

- Gey, F.K., Puska, P., Jordan, P. and Moser, U.K. (1991). Inverse correlation between vitamin E and mortality from ischaemic heart disease in cross-cultural epidemiology. *American Journal of Clinical Nutrition* 53: 3263-3345.
- Gidez, L.I., Miller, G.J. and Burstein, M. (1982). Separation and quantitation of subclasses of human plasma high density lipoproteins by a simple precipitation procedure. *Journal of Lipid Research* 23: 1206-1223.
- Glueck, C.J., Taylor, H.L., Jacobs, D., Morrison, J.A., Beaglehole, R. and Williams, O.D. (1980). Plasma HDL cholesterol: association with measurement of body mass. The Lipid Research Clinics Program Prevalence Study. *Circulation* 62 (Suppl.IV): 62-69.
- Goddard, P. and Hill, M.J. (1972). Degradation of steroids by intestinal bacteria. IV. The aromatisation ring A. *Biochimica et Biophysica Acta* 280: 336-342.
- Goddard, P. and Hill, M.J. (1974). The *in vivo* metabolism of cholesterol by gut bacteria in the rat and guinea pig. *Journal of Steroid Biochemistry* 5: 569.
- Goddard, P., Fernandez, F., West, B., Hill, M.J. and Barnes, P. (1975). The nuclear dehydrogenation of steroids by intestinal bacteria. *Journal of Medical Microbiology* 8: 429.
- Goel, K.M., House, F. and Shanks, R.A. (1978). Infant feeding practices among immigrants in Glasgow. *British Medical Journal* 2: 1181-1183.
- Goel, K.M., Logan, R.W., House, F., Connel, M.D., Stevens, E., Watson, W.H. and Bulloch, C.B. (1978). The prevalence of haemoglobinopathies, nutritional iron and folate deficiencies in native and immigrant children in Glasgow. *Health Bulletin, UM* 36(4): 176-183.
- Goel, K.M., Sweet, A.M., Logan, R.W., Warren, J.M., Arneil, G.C. and Shanks, R.A. (1976). Florid and subclinical rickets among immigrant children in Glasgow. *Lancet* i: 1141-1145.
- Goldberg, M.J., Smith, J.W. and Nichols, R.L. (1977). Comparison of the fecal microflora of Seventh-Day Adventists with individuals consuming a general diet. *Annals of Surgery* 186: 97.
- Goldin, B.R., Adlercreutz, H. and Gorbach, S.L. (1986). The relationship between oestrogen levels and diets of Caucasian American and Oriental immigrant women. *American Journal of Clinical Nutrition* 44: 945-953.
- Goldin, B.R., Aldercreutz, H.D., Sherwood, L., Gorbach, S.L., Warram, M.D., Dwyer, J.T., Swenson, M.S. and Woods, M.N. (1982). Estrogen excretion patterns and plasma levels in vegetarian and omnivorous women. *New England Journal of Medicine* 307(25): 1542-1547.
- Gotto, A.M. (1991). Cholesterol intake and serum cholesterol level. *New England Journal of Medicine* 324(13): 912-913.

- Gray, G., Pike, M.C. and Hirayana, T. (1982). Diet and hormone profiles in teenage girls in four countries at different risk for breast cancer. *Preventive Medicine* 11: 108-113.
- Gray, G.E., Pike, M.C. and Henderson, B.E. (1979). Breast cancer incidence and mortality rates in different countries in relation to known risk factors and dietary practices. *British Journal of Cancer* 39: 1-7.
- Gray, G.E., Pilse, M.C. and Henderson, B.E. (1979). Breast cancer incidence and mortality rates in different countries in relation to known risk factors and dietary practices. *British Journal of Cancer* 39: 1-7.
- Gregory, J., Foster, K., Tyler, H. and Wiseman, M. (1990). *The Dietary and Nutritional Survey of British Adults*. London: HMSO.
- Grimsley, M. and Bhat, A. (1989). Health. In: *Britain's Black Population* (Bhat, A., Carr-Hill, R., Ohri, S., eds). Aldershot: Gower.
- Grindulis, H., Scott, P.H., Belton, N.R. and Wharton, B.A. (1986). An association of anaemia with poor vitamin C status in otherwise adequately nourished Asian toddlers: A case for combined prophylaxis. *Proceedings of the Nutrition Society* 45: 22A.
- Grindulis, H., Scott, P.H., Belton, N.R. and Wharton, B.A. (1986). Combined deficiency of iron and vitamin D in Asian toddlers. *Archives of Disease in Childhood* 61: 843-848.
- Grundy, S.M. (1984). Pathogenesis of hyperlipoproteinemia. *Journal of Lipid Research* 25: 1611-1618.
- Grundy, S.M., Ahrens, E.H. and Miettinen, T.A. (1965). Quantitative isolation and gas-liquid chromatographic analysis of total faecal bile acids. *Journal of Lipid Research* 6: 397-410.
- Gupta, S.P., Siwach, S.B. and Gupta, M.S. (1979). Hypertension and blood pressure trends in the general population of Haryana (based on total communal surveys). *Journal of the Association of Physicians of India* 27: 119-126.
- Hack, M., Breslau, N., Weissman, B., Aram, D., Klein, N. and Borawski, E. (1991). Effect of very low birth weight and subnormal head size on cognitive abilities at school age. *New England Journal of Medicine* 325(4): 231-237.
- Haenszel, W. (1961). Cancer mortality among the foreign born in the United States. *Journal of the National Cancer Institute* 26: 37.
- Haffner, S.M., Stern, M.P., Hazuda, H.P., Rosenthal, M. and Knapp, J.A. (1986). The role of behavioural variables and fat patterning in explaining ethnic differences in serum lipids and lipoproteins. *American Journal of Epidemiology* 123: 830-839.

- Hagerty, M.A., Howie, B.J., Tan, S. and Shultz, T.D. (1988). Effect of low and high fat intakes on the hormonal milieu of premenopausal women. *American Journal of Clinical Nutrition* 48: 653-659.
- Haines, A.P., Chakrabarti, R., Fisher, D., Meade, T.W., North, W.R.S. and Stirling, Y. (1980). Haemostatic variables in vegetarians and non-vegetarians. *Thrombosis Research* 19: 139-148.
- Haines, A.P., Imeson, J.D. and Meade, T.W. (1987). Skinfold thickness and cardiovascular risk factors. *American Journal of Epidemiology* 126: 86-94.
- Hallberg, L. (1981). Bioavailability of dietary iron in man. *Annual Review of Nutrition* 1: 123-147.
- Hämäläinen, E., Adlercreutz, H., Puska, P. and Pietnen, P. (1984). Diet and serum sex hormones in healthy men. *Journal of Steroid Biochemistry* 20: 459-464.
- Hardinge, M.G. and Stare, F.J. (1954). Nutritional status of vegetarians. *American Journal of Clinical Nutrition* 2: 73-82.
- Harris, R.J., Armstrong, D., Al, R. and Loynes, A. (1983). A nutritional survey of Bangladeshi children aged under 5 years in the London Borough of Tower Hamlets. *Archives of Diseases of Childhood* 58: 428-432.
- Harris, W.S. (1989). Fish oils and plasma lipid and lipoprotein metabolism in humans: a critical review. *Journal of Lipid Research* 30: 785-807.
- Haskey, J. (1988). The ethnic minority populations of Great Britain: their size and characteristics. *Population Trends* 54: 29-31.
- Haskey, J. (1989). Families and households of the ethnic minority and white populations of Great Britain. *Population Trends* 57: 8-19.
- Haskey, J. (1990). The ethnic minority populations of Great Britain: estimates of ethnic group and country of birth. *Population Trends* 60: 35-38.
- Heath, D.A. (1983). Thoughts on the aetiology of vitamin D deficiency in Asians. *Postgraduate Medical Journal* 59: 649-651.
- Hegsted, D.M. (1986). Serum-cholesterol response to dietary cholesterol: a revaluation. *American Journal of Clinical Nutrition* 44: 299-305.
- Hegsted, D.M., McGandy, R.B., Myers, M.L. and Stare, F.J. (1965). Quantitative effects of dietary fat on serum cholesterol in man. *American Journal of Clinical Nutrition* 17: 281-295.
- Heiss, G., Tamir, I., Davis, C.E. *et al.* (1980). Lipoprotein cholesterol distributions in selected North American populations: The Lipid Research Clinics Program Prevalence Study. *Circulation* 61: 302-315.

- Helman, A.D. and Darton-Hill, I. (1987). Vitamin and iron status in new vegetarians. *American Journal of Clinical Nutrition* 45: 785-789.
- Helmrich, S.P., Shapiro, S. and Rosenberg, L. (1983). Risk factors for breast cancer. *American Journal of Epidemiology* 115: 241-245.
- Herbert, V.D. and Colman, N. (1988). Folic acid and vitamin B₁₂. In: *Modern Nutrition in Health and Disease* (Shils, M.E. and Young, V.R., eds). Philadelphia: Lea and Febiger.
- Herrmann, W., Biermann, J., Lindhofer, H.G. and Kostner, G. (1989). Modification of atherogenic risk factor Lp(a) by supplementary fish oil administration in patients with moderate physical training. *Medizinische Klinik* 84: 429-433.
- Higginson, J. and Muir, C.S. (1979). Environmental carcinogenesis: Misconception and limitations to cancer control. *Journal of the National Cancer Institute* 63: 1291-1298.
- Hill, M.J. (1971). The effect of some factors on the fecal concentration of acid steroids, neutral steroids and urobilins. *Journal of Pathology* 104: 239.
- Hill, M.J. (1975). The role of colon anaerobes in the metabolism of bile acids and steroids, and its relation to colon cancer. *Cancer* 36: 2387.
- Hill, M.J. (1986). Cancer of the large bowel. In: *Microbes and Human Carcinogenesis*. London: Edward Arnold.
- Hill, M.J. (1986). Carcinogens, mutagens and tumour promoters produced by microbes. In: *Microbes and Human Carcinogenesis*. London: Edward Arnold.
- Hill, M.J. and Aries, V.C. (1971). Faecal steroid composition and its relationship to cancer of the large bowel. *Lancet* i: 95-100.
- Hill, M.J., Draser, B.S., Aries, V.C., Crowther, J.S., Hawksworth, G.B. and Williams, R.E.O. (1971). Bacteria and aetiology of cancer of large bowel. *Lancet* i: 95-100.
- Hill, M.J., Taylor, A.J., Thompson, M.H. and Wait, R. (1982). Faecal steroids and urinary volatile phenols in four Scandinavian populations. *Nutrition and Cancer* 4: 67-73.
- Hill, P., Garbaczewski, L., Helman, P., Huskisson, J., Sporangisa, E. and Wynder, E.L. (1980). Diet, lifestyle and menstrual activity. *American Journal of Clinical Nutrition* 33(11): 982-988.
- Hirayama, T. (1978). Epidemiology of breast cancer with special reference to the role of diet. *Preventive Medicine* 7: 173-195.
- Hirayama, T. (1978). Epidemiology of breast cancer with special reference to the role of diet. *Preventive Medicine* 7: 173-195.

- Hirayama, T. (1979). Diet and cancer. *Nutrition and Cancer* 1: 69-81.
- Hirayama, T. (1981). A large scale cohort study on the relationship between diet and selected cancers of digestive organs. In: *Banbury Report*, No.7. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, pp 409-429.
- Hirayama, T. (1985). Diet and cancer: feasibility and importance of prospective cohort study. In: *Diet and Human Carcinogenesis* (Joossens, J.V., Hill, M.J. and Geboers, J., eds). Amsterdam/New York: Elsevier.
- Hirsch, J., Farquhar, J.W., Ahrens, E.H., Peterson, M.L. and Stoffel, W. (1960). Studies of adipose tissue in man: a microtechnic for sampling and analysis. *American Journal of Clinical Nutrition* 8: 499-511.
- Hjermann, I., Holme, I. and Leren, P. (1986). Oslo Study Diet and Antismoking Trial. Results after 102 months. *American Journal of Medicine* 80: 7-11.
- Hjermann, I., Velve-Byre, K., Holme, I. and Leren, P. (1981). Effect of diet and smoking intervention on the incidence of coronary heart disease. Report from the Oslo Study Group of a randomised trial in healthy men. *Lancet* ii: 1303-1310.
- Hodgkin, P., Hine, P.M., Kay, C.H., Lumb, G.A. and Stanbury, S.W. (1973). Vitamin D deficiency in Asians at home and in Britain. *Lancet* ii: 167-172.
- Holland, B., Unwin, J.D. and Buss, D.H. (1988). *Cereals and Cereal Products*. The Supplement to McCance and Widdowson's "The Composition of Foods". London: Royal Society of Chemistry and MAFF.
- Holmes, A.M., Enoch, B.A., Taylor, J.L. and Jones, M.E. (1973). Occult rickets and osteomalacia amongst the Asian immigrant population. *Quarterly Journal of Medicine* 42: 125-149.
- Honeyman, M.M., Bahl, L., Marshall, T. and Wharton, B.A. (1987). Consanguinity and fetal growth in Pakistani Moslems. *Archives of Diseases of Childhood* 62: 231-235.
- Hopkins, G.J. and Carroll, K.K. (1979). Relationship between amount and type of dietary fat in promotion of mammary carcinogenesis induced by 7,12-dimethylbenz(α)anthracene. *Journal of the National Cancer Institute* 62: 1009-1012.
- Hornstra, G., van Houwelingen, A.C., Simonis, M. and Gerrard, J.M. (1989). Fatty acid composition of umbilical arteries and veins: possible implications for the fetal EFA-status. *Lipids* 2(6): 511-517.
- Horwitt, M.K., Harper, C.C. and Century, B. (1959). Effects of dietary fats on human erythrocyte pattern. *Journal of Clinical Investigation* 42: 675-686.
- Howell, M.A. (1975). Diet as an etiological factor in the development of cancers of the colon and rectum. *Journal of Chronic Diseases* 28: 67-80.

- Hughes, L.O. and Cruikshank, J.K. (1989). Ischaemic heart disease in people of Indian subcontinent origin. In: *Ethnic Factors in Health and Disease* (Cruikshank, J.K. and Beevers, D.G., eds). London: Wright.
- Hughes, L.O., Wojciechowski, A.D. and Raftery, E.B. (1990). Relationship between plasma cholesterol and coronary artery disease in Asians. *Atherosclerosis* 83: 15-20.
- Hunt, S. (1976). The food habits of Asian immigrants. In: *Getting the Most out of Food*. Van de Berghs and Jurgens Ltd, pp 15-51.
- Hunt, S.P., O'Riordan, J.L.H., Window, J. and Trusswell, A.S. (1976). Vitamin D status in different sub-groups of British Asians. *British Medical Journal* 2: 1351-1354.
- Hussain, M.A. and Wadsworth, G.R. (1967). Nutritional status of Asian infants. *Proceedings of the Nutrition Society* 26: 212-218.
- Hylemon, P.B. and Glass, T.L. (1983). Biotransformation of bile acids and cholesterol by the intestinal microflora. In: *Human Intestinal Microflora in Health and Disease* (Hentges, D.J., ed.). New York: Academic Press.
- IARC, Large Bowel Cancer Group (1982). Second IARC International Collaborative Study on diet and large bowel cancer in Denmark and Finland. *Nutrition and Cancer* 4: 3-79.
- Illingworth, D.R., Sundberg, E.E., Becker, N., Connor, W.E. and Alanpovic, P. (1981). Influence of saturated, monounsaturated and n-6 PUFA on LDL metabolism in man. *Arteriosclerosis* 1: 380.
- Ingram, D., Nottage, E., Ng, S., Sparrow, L., Roberts, A. and Willcox, D. (1990). Obesity and breast disease: the role of female sex hormones. *Cancer* (in press).
- International Nutritional Anaemia Consultative Group (1982). *The Effects of Cereals and Legumes on Iron Availability*. Washington: Nutrition Foundation.
- Ip, C. (1987). Fat and essential fatty acid in mammary carcinogenesis. *American Journal of Clinical Nutrition* 45: 218-224.
- Ip, C., Yip, P. and Bernadio, L.L. (1980). Role of prolactin in the promotion of dimethylbenzanthracene-induced mammary tumours by dietary fat. *Cancer Research* 40: 374-378.
- Islam, N., Khan, M. and Latif, Z.A. (1983). Hypertension in the rural population of Bangladesh - a preliminary survey. *Bangladesh Medical Research Council Bulletin* 9: 11-14.
- Jacobs, A., Water, W.E., Campbell, H. and Barrow, A. (1969). A random sample from Wales. *British Journal of Haematology* 17: 581.

- Jacobs, C. and Dwyer, J.T. (1988). Vegetarian children: appropriate and inappropriate diets. *American Journal of Clinical Nutrition* 48(3): 811-819.
- Jain, M., Cook, G.M., Davis, F.G., Grace, M.G., Howe, G.R. and Miller, A.B. (1980). A case-control study of diet and colorectal cancer. *International Journal of Cancer* 26: 757-768.
- Janerich, D.T. and Hoff, M.B. (1982). Evidence for a crossover in breast cancer risk factors. *American Journal of Epidemiology* 116: 737-742.
- Jensen, O.M. (1986). The epidemiology of large bowel cancer. In: *Diet, Nutrition and Cancer: A Critical Evaluation*, Vol.I - Macronutrients and Cancer (Reddy, B.S. and Cohen, L.A., eds). Florida: CRC Press.
- Jensen, O.M., Sigtryggsson, P., Nguyen-Dinh, X., Bolander, A.M., Vercelli, M. and MacLennan, R. (1980). Large bowel cancer in married couples in Sweden. A follow-up study. *Lancet* i: 1161-1163.
- Jenson, O.M. (1983). Cancer risk among Danish male Seventh Day Adventists and other temperance society members. *Journal of the National Cancer Institute* 70(6): 1011-1014.
- Jivani, S.K.M. (1978). The practice of infant feeding among Asian immigrants. *Archives of Diseases in Childhood* 53: 69-73.
- Jordan, L. and Waine, B. (1986). Women's income in and out of employment. *Critical Social Policy* 6(3): 63-78.
- Kahn, R.H., Phillips, R.L., Snowdon, D.A. and Choi, W. (1984). Association between reported diet and all cause mortality: twenty-one year follow-up on 27,530 adult Seventh Day Adventists. *American Journal of Epidemiology* 119: 775-787.
- Kalka, I. (1988). The changing food habits of Gujaratis in Britain. *Journal of Human Nutrition and Dietetics* 1: 389-335.
- Kaluzny, M.A., Duncan, L.A., Merrit, M.V. and Epps, D.E. (1985). Rapid separation of lipid classes in high yield and purity using bonded phase columns. *Journal of Lipid Research* 26: 135-143.
- Karmali, R.A. (1987). Fatty acids: inhibition. *American Journal of Clinical Nutrition* 45: 225-229.
- Karseras, J. and Hopkins, J. (1987). *British Asians - Health in the Community*. London: John Wiley and Son.
- Kay, R.M. (1982). Dietary fiber. *Journal of Lipid Research* 23: 221.
- Kekki, M. (1980). Lipoprotein-lipase action determining plasma high density lipoprotein cholesterol in adult normolipemics. *Atherosclerosis* 37: 3-50.

- Kern, F. (1991). Normal plasma cholesterol in an 88-year old man who eats 25 eggs a day. *New England Journal of Medicine* 324(13): 896-899.
- Kessie, G. and Nair, P.P. (1985). Inhibition by bile acids of phenobarbital-induced forms of hepatic glutathione sulfotransferase. *Federation Proceedings* 44: 1859 (Abstract).
- Key, T.J.A. and Pike, M.C. (1988). The role of oestrogens and progesterone in the epidemiology and prevention of breast cancer. *European Journal of Cancer and Clinical Oncology* 24(1): 29-43.
- Key, T.J.A., Roe, L., Thorogood, M., Moore, J.W., Clark, G.M.G. and Wang, D.Y. (1990). Testosterone, sex hormone-binding globulin, calculated free testosterone, and oestradiol in male vegans and omnivores. *British Journal of Nutrition* 64(1): 111-119.
- Keys, A. (1970). Coronary heart disease in seven countries. *Circulation* 41: 1-211.
- Keys, A. (1984). Serum cholesterol response to dietary cholesterol. *American Journal of Clinical Nutrition* 40: 351-359.
- Keys, A., Anderson, J.T. and Grande, F. (1965). Serum cholesterol response to changes in the diet. IV: Particular saturated fatty acids in the diet. *Metabolism* 14: 776-787.
- Kidwell, W.R., Knazek, R.A., Vonderhaar, B.K. and Lasonczy, I. (1982). Effects of unsaturated fatty acids on the development and proliferation of normal and neoplastic breast epithelium. In: *Molecular Interactions of Nutrition and Cancer* (Arnott, M.S., van Eyes, J. and Yang, Y., eds). New York: Raven Press.
- Kinlen, L.J. (1982). Meat and fat consumption and cancer mortality: A study of strict religious orders in Britain. *Lancet* i: 946-949.
- Kinlen, L.J., Hermon, C. and Smith, P.G. (1983). A proportionate study of cancer mortality among members of a vegetarian society. *British Journal of Cancer* 48: 355-361.
- Kirby, R.W., Anderson, J.W., Sieling, B., Rees, E.D., Chen, W.J.L., Miller, R.E. and Kay, R.M. (1981). Oat bran intake selectively lowers serum LDL-cholesterol concentrations of hypocholesterolemic men. *American Journal of Clinical Nutrition* 34: 824.
- Klasky, A.L., Armstrong, M.A., Friedman, G.D. and Hiatt, R.A. (1988). The relations of alcoholic beverage use to colon and rectal cancer. *American Journal of Epidemiology* 128: 1007-1015.
- Knox, E.G. (1977). Food and disease. *British Journal of Preventive and Social Medicine* 31: 71-80.

- Kohlmeier, L., Rehm, J. and Hoffmeister, H. (1990). Lifestyle and trends in world-wide breast cancer rates. In: *Trends in Cancer Mortality in Industrial Countries*. New York: New York Academy of Sciences.
- Kolonel, L.N., Hankin, J.H. and Nomura, A.M.Y. (1986). Multi-ethnic studies of diet, nutrition and cancer in Hawaii. In: *Diet, Nutrition and Cancer*. Proceedings of 16th International Symposium of the Princess Takamatsu Cancer Research Fund. Tokyo: Japanese Scientific Society Press.
- Kolonel, L.N., Hankin, J.H., Lee, J., Chu, S.Y., Nomura, A.M. and Hinds, M.W. (1981). Nutrient intakes in relation to cancer incidence in Hawaii. *British Journal of Cancer* 44: 332-339.
- Krauss, R.M. (1982). Regulation of high density lipoprotein levels. In: *Symposium on Lipid Disorders*. Medical Clinics of North America Vol.66, No.2. London: W.B. Saunders.
- Krauss, R.M., Lindgren, F.T., Wingerd, J., Bradley, D.D. and Ramcharan, S. (1979). Effects of estrogens and progestins on high density lipoproteins. *Lipids* 14: 113-118.
- Kritchevsky, D., Weber, M.M. and Klurfeld, D.M. (1984). Dietary fat versus calorie content in initiation and promotion of 7-12 dimethylbenz(α)anthracene in mammary tumourigenesis in rats. *Cancer Research* 44: 3174-3177.
- Kune, S., Kune, G.A. and Watson, L.F. (1987). Case-control study of dietary etiological factors: The Melbourne Colorectal Cancer Study. *Nutrition and Cancer* 9: 21-42.
- Kuratsune, M., Honda, T., Englyst, H.N. and Cummings, J.H. (1986). Dietary fibre in the Japanese diet as investigated in connection with colon cancer risk. *Japanese Journal of Cancer Research* 77: 736-738.
- Kurihara, M., Aoki, K. and Tominaga, S. (1984). *Cancer Mortality Statistics in the World*. Nagoya, Japan: University of Nagoya Press.
- Kuusi, T., Ehnholm, C., Huttunen, J.K. *et al.* (1985). Concentration and composition of serum lipoproteins during a low-fat diet at two levels of polyunsaturated fat. *Journal of Lipid Research* 26: 360-367.
- Kuusi, T., Sarinen, P. and Nikkilä, E.A. (1980). Evidence for the role of hepatic endothelial lipase in the metabolism of plasma high density lipoproteins in man. *Atherosclerosis* 36: 589-593.
- La Vecchia, C., Decarli, A., DiPietro, S., Francheschi, S., Negri, E. and Parazzini, F. (1985). Menstrual cycle patterns and the risk of breast disease. *European Journal of Cancer and Clinical Oncology* 21: 417-421.

- La Vecchia, C., Franceschi, S. and Gallus, J.J. (1982). Prognostic features of endometrial cancers in oestrogenusers and obese women. *American Journal of Obstetrics and Gynaecology* 144: 387-390.
- Labour Force Survey (1985). London: OPCS.
- Labour Force Survey (1987). London: OPCS.
- Lai, C.S., Hopwood, L.E. and Swartz, H.M. (1980). Electron spin resonance studies of changes in membrane fluidity of Chinese hamster ovary cells during the cell cycle. *Biochimica et Biophysica Acta* 602: 117-126.
- Lechtig, A., Yarborough, C., Delgado, H., Habicht, J.P., Martorell, R. and Klein, R.E. (1975). Influence of maternal nutrition on birth weight. *American Journal of Clinical Nutrition* 28: 1223-1233.
- Lee, H.P., Gourley, L., Duffy, S.W., Estene, J., Lee, J. and Day, N.E. (1991). Dietary effects on breast-cancer risk in Singapore. *Lancet* 337: 1197-1200.
- Lemon, R.R. and Walden, R.T. (1966). Death from the respiratory system disease among Seventh Day Adventist man. *Journal of the American Dietetic Association* 198: 117-126.
- Lemon, R.R., Walden, R.T. and Woods, R.W. (1964). Cancer of the lung and mouth in Seventh-Day Adventists. *Cancer* 17: 486-497.
- Leonard, P.J. and Losowsky, M.S. (1971). Effect of alpha-tocopherol administration on red cell survival in vitamin E-deficient human subjects. *American Journal of Clinical Nutrition* 24: 388-393.
- Lepage, G. and Roy, C.C. (1986). Direct transesterification of all classes of lipids in a one-step reaction. *Journal of Lipid Research* 27: 114-120.
- Lew, E.A. and Garfinkel, L. (1979). Variations in mortality and weight among 750,000 men and women. *Journal of Chronic Diseases* 32: 563-576.
- Liu, C.F., Carlson, S.E., Rhodes, P.G., Rao, V.S. and Meydrech, E.F. (1987). Increase in plasma phospholipid docosahexaenoic and eicosapentaenoic acids as a reflection of their intake and mode of administration. *Paediatric Research* 22: 292-296.
- Lo, C.W., Paris, P.W. and Hobek, M.F. (1986). Indian and Pakistani immigrants have the same capacity as Caucasians to produce vitamin D in response to ultra-violet irradiation. *American Journal of Clinical Nutrition* 44: 683-685.
- Logan, R.L., Thomson, M.R. and Riemersma, R.A. (1984). Risk factors for ischaemic heart disease in normal men aged 40. *Lancet* i: 949-955.

- Longcope, C., Gorbach, S., Goldin, B., Woods, M., Dwyer, J., Morrill, A. and Warram, J. (1987). The effect of a low fat diet on estrogen metabolism. *Journal of Clinical Endocrinology and Metabolism* 64(6): 1246-1249.
- Lopes, S.M., Trimbo, S.L., Mascioli, E.A. and Blackburn, G.L. (1991). Human plasma fatty acid variations and how they are related to dietary intake. *American Journal of Clinical Nutrition* 53: 628-637.
- Loscalzo, J. (1990). Lipoprotein (a). A unique risk factor for atherothrombotic disease. *Arteriosclerosis* 10(5): 672-679.
- Lowenfels, A.B. (1983). Is increased cholesterol excretion the link between low serum cholesterol and colon cancer? *Nutrition and Cancer* 4: 280-284.
- Lowry, P.J., Glover, D.R., Mace, J.E. and William, A.L. (1984). Coronary artery disease in Asians in Birmingham. *British Heart Journal* 52: 610-613.
- Lubin, F., Ruder, A.M., Wax, Y. and Modan, B. (1985). Overweight and changes in weight throughout adult life in breast cancer aetiology. *American Journal of Epidemiology* 122: 579-588.
- Lubin, J.H., Burns, P.E., Blot, W.J., Ziegler, R.G., Lees, A.W. and Fraumeni, J.F. (1981). Dietary factors and breast cancer risk. *International Journal of Cancer* 28: 685-689.
- Luthera, M.S. (1988). In: *Britain's Black Population. A New Perspective*, 2nd edition (Bhat, A., Carr-Hill, R. and Ohri, S., eds). The Radical Statistical Race Group. Aldershot: Gower Publishing Co. Ltd.
- Lyon, J.L. and Sorensen, A.W. (1978). Colon cancer in low risk population. *American Journal of Clinical Nutrition* 31: 5227-5230.
- Lyon, J.L., Gardner, J.W. and West, D.W. (1980). Cancer risk and life style: cancer among Mormons from 1967-1975. In: *Cancer Incidence in Defined Populations* (Cairns, J., Lyon, J.L. and Skolnick, N., eds) Banbury Report No.4. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Lyon, J.L., Mahoney, A.W., West, D.W., Gardner, J.W., Smith, K.R., Sorenson, A.W. and Stanish, W. (1987). Energy intake: Its relationship to colon cancer risk. *Journal of the National Cancer Institute* 78: 853-861.
- Macdonald, I.A., Bokkenheuser, V.D., Winter, J., McLernon, A.M. and Mosbach, E.H. (1983). Degradation of steroids in the human gut. *Journal of Lipid Research* 24: 675.
- Macfarlane, G.T., Cummings, J.H. and Allison, C. (1986). Protein degradation by human intestinal bacteria. *Journal of General Microbiology* 132: 1647-1656.
- MacPhail, A.P., Bothwell, T.H. and Torrance, J.D. (1981). Fe nutrition in Indian women at different ages. *South African Medical Journal* 59: 939-942.

- Macquart-Moulin, G., Riboli, E., Cornée, J., Charnay, B., Berthezéne, P. and Day, N. (1986). Case-control study on colorectal cancer and diet in Marseille. *International Journal of Cancer* 38: 183-191.
- Malhotra, S.L. (1967a). Geographical distribution of gastrointestinal cancers in Indians with special reference to causation. *Gut* 8: 361-372.
- Malhotra, S.L. (1967b). Epidemiology of ischaemic heart disease in India with special reference to causation. *British Heart Journal* 29: 895-905.
- Manouses, O., Day, N.E., Trichopoulos, D., Gerovassilis, F., Tzonou, A. and Polychronopoulou, A. (1983). Diet and colorectal cancer: a case control study in Greece. *International Journal of Cancer* 32: 1-5.
- Margetts, B.M., Beilin, L.J., Vandongen, R. and Armstrong, B.K. (1986). Vegetarian diet in mild hypertension: a randomised controlled trial. *British Medical Journal* 293: 129-133.
- Marine, N., Vinik, A.I., Edelstein, I. and Jackson, W.P.U. (1969). Diabetes, hyperglycaemia and glycosuria among Indians, Malays and Africans (Bantu) in Cape Town, South Africa. *Diabetes* 18: 840-857.
- Marmot, M.G. (1984). *Immigrant Mortality in England and Wales 1970-78*. OPCS Studies of Medical Population Subjects No.47. London: HMSO.
- Marmot, M.G., Adelstein, A.M. and Bulusu, L. (1984). Lessons from the study of immigrant mortality. *Lancet* i: 1455-1457.
- Marsh, A.G., Sanchez, T.V., Chaffee, F.L., Mayor, G.H. and Mickelsen, O. (1983). Bone mineral mass in adult lacto-vegetarian and omnivorous males. *American Journal of Clinical Nutrition* 3: 453-456.
- Marsh, A.G., Sanchez, T.V., Michelsen, O., Chaffee, F.L. and Fagal, S.M. (1988). Vegetarian lifestyle and bone mineral density. *American Journal of Clinical Nutrition* 48: 837-841.
- Marsh, A.G., Sanchez, T.V., Michelsen, O., Keiser, J. and Mayor, G. (1980). Cortical bone density of adult lactovegetarians and omnivorous women. *Journal of the American Dietetic Association* 76: 148-150.
- Marx, J.L. (1983). Do tumour promoters affect DNA after all? *Science* 219: 158-159.
- Mather, H.M. and Keen, H. (1985). The Southall diabetes survey: prevalence of known diabetes in Asians and Europeans. *British Medical Journal* 291: 1081-1084.
- Matheson, L.M., Dunnigan, M.G., Hole, D. and Gillis, C.R. (1985). Incidence of colorectal, breast and lung cancer in Scottish Asian population. *Health Bulletin* 43: 245-249.

- Mathews, J.H. and Wood, J.K. (1984). Megaloblastic anaemia in vegetarian Asians. *Clinical Laboratory Haematology* 6: 1-7.
- Mattson, F.H. and Grundy, S.M. (1986). Comparison of dietary saturated, mono-unsaturated and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. *Journal of Lipid Research* 26: 194-202.
- Mattson, F.H., Hollenback, E.J. and Kligman, A.M. (1975). Effect of hydrogenated fat on plasma cholesterol and triglyceride levels of man. *American Journal of Clinical Nutrition* 28: 726-731.
- McAvoy, B. (1990). Women's Health. In: *Health Care for Asians* (McAvoy, B.R. and Donaldson, L.J., eds). Oxford: Oxford University Press.
- McAvoy, B.R. and Raza, R. (1988). Asian women: (i) Contraceptive knowledge, attitudes and usage. (ii) Contraceptive services and cervical cytology. *Health Trends* 20: 11-17.
- McBurney, M.I., Horvath, P.J., Jeraci, J.L. and Van Soet, P.J. (1985). Effect of *in vivo* fermentation using human faecal inoculum on the water holding capacity of dietary fibre. *British Journal of Nutrition* 53: 17-24.
- McCay, D., Bannerjee, S.C., Ghosal, L.M., Dutta, M.M. and Roy, C. (1916). Observations on the sugar of the blood and the sugar in the urine in varying conditions of health in the Bengali. *Indian Journal of Medical Research* 4: 1-27.
- McCormick, J. and Skrabanek, P. (1988). Coronary heart disease is not preventable by population interventions. *Lancet* ii: 839-841.
- McDonald, B.E., Gerrard, J.M., Bruce, V.M. and Corner, E.J. (1989). Comparison of the effect of canola oil and sunflower oil on plasma lipids and lipoproteins and on *in vivo* thromboxane A₂ and prostacyclin production in healthy young men. *American Journal of Clinical Nutrition* 50: 1382-1388.
- McFadyen, I.R. (1985). Fetal growth. In: *Progress in Obstetrics and Gynaecology*, Vol.5 (Studd, J., ed.). Edinburgh: Churchill Livingstone, pp 55-77.
- McFadyen, I.R., Campbell-Brown, M., Abraham, R., North, W.R.S. and Haines, A.P. (1984). Factors affecting birthweight in Hindus, Moslems and Europeans. *British Journal of Obstetrics and Gynaecology* 91: 968-972.
- McGandy, R.B., Hegsted, D.M. and Myers, M.L. (1970). Use of semi-synthetic fats in determining effects of specific dietary fatty acids on serum lipids in man. *American Journal of Clinical Nutrition* 23: 1288-1298.
- McGill, H.C. (1979). The relationship of dietary cholesterol to serum cholesterol and atherosclerosis in man. *American Journal of Clinical Nutrition* 32: 2664-2702.
- McKeigue, P.M., Adelstein, A.M., Marmot, M.G., Henly, P.J., Owen, R.W., Hill, M.J. and Thompson, M.H. (1989). Diet and fecal steroid profile in a South

Asian population with a low colon-cancer rate. *American Journal of Clinical Nutrition* 50: 151-154.

McKeigue, P.M., Adelstein, A.M., Shipley, M.J., Riemersma, R.A., Marmot, M.G., Hunt, S.P., Butler, S.M. and Turner, P.R. (1985). *Diet and risk factors for coronary heart disease in Asians in North West London*. *Lancet* ii: 1086-1089.

McKeigue, P.M. and Marmot, M.G. (1988). Mortality from coronary heart disease in Asian communities in London. *British Medical Journal* 297: 903.

McKeigue, P.M., Marmot, M.G., Syndercombe, Count Y.D., Colties, D.E., Rahman, S. and Riemersma, R.A. (1988). Diabetes, hyperinsulinaemia and coronary risk factors in Bangladeshis in East London. *British Heart Journal* 60: 390-396.

McKeigue, P.M., Miller, G.J. and Marmot, M.G. (1989). Coronary heart disease in South Asians overseas - A review. *Journal of Clinical Epidemiology* 42(7): 597-609.

McKenzie, S.B. (1988). *Textbook of Haematology*. Philadelphia: Lea and Febiger, p.108.

McKeown-Eyssen, G.E. and Bright-See, E. (1984). Dietary factors in colon cancer: international relationships. *Nutrition and Cancer* 6: 160-170.

McKeown-Eyssen, G.E. and Bright-See, E. (1985). Dietary factors in colon cancer: international relationships. An update. *Nutrition and Cancer* 7: 251-253.

McLean, I., Walter, R.L., Davies, P.S., Hill, M.J., Drasar, B.S. and Southgate, D.A.T. (1977). The effects of two dietary fiber supplements on gastrointestinal transit, stool weight and frequency, and bacterial flora and fecal bile acids in normal subjects. *Metabolism* 26: 117.

McManus, M.J. and Welsch, C.W. (1984). The effect of oestrogen, progesterone, thyroxine and human placental lactogen on DNA synthesis of human breast ductal epithelium maintained in athymic nude mice. *Cancer* 54: 1920-1927.

McMichael, A.J. and Potter, J.D. (1980). Reproduction, endogenous and exogenous sex hormones and colon cancer: a review and hypothesis. *Journal of the National Cancer Institute* 65: 1201.

McMichael, A.J., McCall, M.G., Hartshore, J.M. and Woodlings, T.L. (1980). Patterns of gastro-intestinal cancer in European immigrants to Australia: the role of dietary change. *International Journal of Cancer* 25: 431.

McMichael, A.J., Potter, J.D. and Hetzel, B.S. (1979). Time trends in colorectal cancer mortality in relation to food and alcohol consumption: US, UK, Australia, New Zealand. *International Journal of Epidemiology* 8: 295-303.

McMurchie, E.J., Margetts, B.M., Beilin, L.J., Croft, K.D., Vandongen, R. and Armstrong, B.K. (1984). Dietary induced changes in the fatty acid composition

of human cheek cell phospholipids: correlation with changes in the dietary polyunsaturated/saturated fat ratio. *American Journal of Clinical Nutrition* 39: 975-980.

- McNeil, G. (1985). Birth weight, feeding practices and weight for age of Punjabi children in the U.K. and in the rural Punjab. *Human Nutrition : Clinical Nutrition* 39C: 69-72.
- Mehra, N.K., Taneja, V., Kailash, S., Raizada, N. and Vaidya, M.C. (1986). Distribution of HLA antigens in a sample of the North Indian population. *Tissue Antigens* 27: 64-74.
- Mendelsohn, D. and Mendelsohn, L. (1989). Effect of polyunsaturated fat on regression of atheroma in the non-human primate. *South African Medical Journal* 76: 371-373.
- Mensink, R.P. and Katan, M.B. (1989). Effect of a diet enriched with monounsaturated or polyunsaturated fatty acids on levels of low density and high density lipoprotein cholesterol in healthy men and women. *New England Journal of Medicine* 321: 436-441.
- Meyer, P.D., Denbesten, L. and Mason, E.E. (1979). The effects of a high fibre diet on bile acid pool size, bile acid kinetics and biliary lipid secretory rates in the morbidly obese. *Surgery* 85: 311-316.
- Miettinen, M., Turpeinen, O., Karvanon, M.J., Elosuo, R. and Paavilainen, E. (1972). Effect of cholesterol-lowering diet on mortality from coronary heart disease and other causes. A twelve-year clinical trial in men and women. *Lancet* ii: 835-838.
- Miettinen, T.A., Gylling, H. and Vanhanen, H. (1988). Serum cholesterol response to dietary cholesterol and apoprotein E phenotype. *Lancet* ii: 1261.
- Miller, A.B. (1978). An overview of hormone-associated cancers. *Cancer Research* 38: 3985-3989.
- Miller, A.B. (1985). Diet, nutrition and cancer: an epidemiological overview. *Journal of Nutrition, Growth and Cancer* 2: 159-171.
- Miller, A.B., Howe, G.R., Jain, M., Craib, K.J.P. and Harrison, L. (1983). Food items and food groups as risk factors in a case-control study of diet and colorectal cancer. *International Journal of Cancer* 32: 155-161.
- Miller, G.J., Alexis, S.D., Beckler, G.L.A. and Byam, N.T.A. (1982). Serum lipoproteins and susceptibility of men of Indian descent to CHD, the St James Survey, Trinidad. *Lancet* ii: 200-203.
- Miller, G.J. and Miller, N.E. (1975). Plasma high density lipoprotein concentration and development of ischaemic heart disease. *Lancet* i: 16-19.

- Miller, G.J., Kirkwood, B.R., Beckles, G.L.A., Alexis, S.D., Carson, D.C. and Byam, N.T.A. (1988). Adult male all-cause cardiovascular and cerebrovascular mortality in relation to ethnic group systolic blood pressure and blood glucose concentration in Trinidad, West Indies. *International Journal of Epidemiology* 17: 62-69.
- Miller, G.J., Kotecha, S., Wilkinson, W.H., Wilkes, H., Stirling, Y., Sanders, T.A.B., Broadhurst, A., Allison, J. and Meade, T.W. (1988). Dietary and other characteristics relevant for coronary heart disease in men of Indian, West Indian and European descent in London. *Atherosclerosis* 70: 63-72.
- Modan, B., Barell, V., Lubin, F., Modan, M., Greenberg, R.A. and Graham, S. (1975). Low-fibre intake as an aetiological factor in cancer of the colon. *Journal of the National Cancer Institute* 55: 15-18.
- Mohrhauer, H. and Holman, R.T. (1963). Alteration of the fatty acid composition of brain lipids by varying levels of dietary essential fatty acids. *Journal of Neurochemistry* 10: 523-530.
- Mohrhauer, H. and Holman, R.T. (1963). Alteration of the fatty acid composition of brain lipids by varying levels of dietary essential fatty acids. *Journal of Neurochemistry* 10: 523-530.
- Moore, J.W., Clark, G.M.G., Bulbrook, R.D., Hayward, J.L., Murai, J.T., Hammond, G.L. and Siiteri, P.K. (1982). Serum concentrations of total and non-protein-bound oestradiol in patients with breast cancer and in normal controls. *International Journal of Cancer* 29: 17-21.
- Moore, J.W. and Bulbrook, R.D. (1988). The epidemiology and function of sex hormone binding globulin. In: *Oxford Reviews of Reproductive Biology*, Vol.10 (Clark, J., ed.). Oxford: Oxford University Press, pp 180-236.
- Moore, R.A., Oppert, S., Eaton, P. and Mann, J.L. (1977). Triglyceride fatty acids confirm change in dietary fat. *Clinical Endocrinology* 7: 143-149.
- Moore, W.E.C. and Holdeman, L.V. (1975). Discussion of current bacteriological investigations of the relationships between intestinal flora, diet and colon cancer. *Cancer Research* 35: 3418.
- Morrison, W.R. and Smith, L.M. (1964). Preparation of fatty acid methyl esters and dimethyl acetals from lipids with boron fluoride-methanol. *Journal of Lipid Research* 5: 600-608.
- Mower, H.F., Ichinotsubo, D., Wang, L.W., Mandel, M., Stemmerman, A., Nomura, A., Heilbrun, L., Kamiyama, S. and Shimada, A. (1982). Fecal mutagens in two Japanese populations with different colon cancer risks. *Cancer Research* 42: 1164.
- MRC Vitamin Study Research Group (1991). Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. *Lancet* 338(8760): 131-137.

- MRFIT Research Group (1982). Multiple Risk Factor Intervention Trial: risk factor changes and mortality results. *Journal of the American Medical Association* **248**: 1465-1477.
- Muir, C., Waterhouse, J., Mack, T., Powell, J. and Whelan, S. (1987). *Cancer Incidence in Five Continents*. V.I.A.R.C. Scientific Publication No.88. Lyon: International Agency for Research on Cancer.
- Mukherjee, A.B. (1973). Pathogenesis of diabetes mellitus. *Journal of the Indian Medical Association* **61**: 1-6.
- Munoz, J. (1984). Fibre and diabetes. *Diabetes Care* **7**: 297-298.
- Myant, N.B. (1990). LDL: origin and metabolism. In: *Cholesterol Metabolism, LDL and the LDL Receptor*. London: Academic Press.
- Nair, P.P. (1988). Role of bile acids and neutral sterols in carcinogenesis. *American Journal of Clinical Nutrition* **48**: 768-774.
- Nair, P.P., Turjiman, N., Goodman, G.T., Guidry, C. and Calkins, B.M. (1984). Diet, nutrition intake and metabolism in populations at high and low risk for colon cancer. Metabolism of neutral sterols. *American Journal of Clinical Nutrition* **40**: 931-936.
- Nanjee, M.N. and Wheeler, M.J. (1985). Plasma free testosterone - is an index sufficient? *Annals of Clinical Biochemistry* **22**: 387-390.
- National Diet - Heart Study Research Group (1968). National Diet - Heart Study final report. *Circulation* **37**(Suppl.1).
- National Institute of Nutrition (1980). *Community Studies using Salt Fortified with Iron*. Annual Report. Hyderabad: NIN, pp 166-169.
- National Research Council (1989). *Diet and Health: Implications to reduce chronic disease risk*. Washington DC: National Academy Press.
- National Research Council (1989). *Recommended Daily Allowances*, 10th edition. Washington DC: National Academy Press.
- Neuringer, M. and Connor, W.E. (1989). *Omega-3 Fatty Acid: Biological Effects and Nutritional Essentiality*. New York: Plenum Publishing.
- Neuringer, M., Connor, W.E., Van Petten, C. and Barstad, L. (1984). Dietary omega-3 fatty acid deficiency and visual loss in infant rhesus monkeys. *Journal of Clinical Investigation* **73**: 272-276.
- Nicoll, A., Miller, N.E. and Lewis, B. (1980). High density lipoprotein metabolism. *Advances in Lipid Research* **17**: 91-116.

- Nielsen, N.H. and Hanson, J.P.H. (1980). Breast cancer in Greenland: selected epidemiological, clinical and histological features. *Journal of Cancer Research and Clinical Oncology* 98: 287-299.
- Nomura, A., Henderson, B.E. and Lee, J. (1978). Breast cancer and diet among the Japanese in Hawaii. *American Journal of Clinical Nutrition* 31: 2020-2025.
- Northfield, T.C. and Hoffman, A.F. (1973). Biliary lipid secretion in gallstone patients. *Lancet* i: 747-748.
- O'Hare, A.E., Utley, W.S., Belton, N.R. *et al.* (1984). Persisting Vit D deficiency in Asian adolescents. *Archives of Diseases in Childhood* 59: 766-770.
- Office of Population Census and Surveys (1981). *Census 1981*. London: HMSO.
- Ohuchi, K. and Levine, L. (1980). α -Tocopherol inhibits 12-O-tetradecanoyl-phorbol-13-acetate-stimulated deacylation of cellular lipids, prostaglandin production and changes in cell morphology of Modin-Darby canine kidney cells. *Biochimica et Biophysica Acta* 619: 11-19.
- Olsson, H., Landin-Olsson, M. and Gullberg, B. (1983). Retrospective assessment of menstrual cycle length in patients with breast cancer, in patients with benign breast disease and in women without breast disease. *Journal of the National Cancer Institute* 70: 17-20.
- Ophir, O., Peer, G., Giland, J., Blum, M. and Avia, A. (1983). Low blood pressure in vegetarians: the possible role of potassium. *American Journal of Clinical Nutrition* 37: 755-762.
- Oser, B. (1965). *Hawks Physiological Chemistry*, 14th edition. New York: McGraw-Hill Publishing Company Ltd.
- Owen, R.W., Thompson, M.H. and Hill, M.J. (1984). Analysis of metabolic profiles of steroids in faeces of healthy subjects undergoing cheenodeoxycholic acid treatment by liquid-gel chromatography and gas-liquid chromatography - mass spectrometry. *Journal of Steroid Biochemistry* 21(5): 593-600.
- Packard, C.J., McKinney, L., Carr, K. and Shepherd, J. (1983). Cholesterol feeding increases low density lipoprotein synthesis. *Journal of Clinical Investigation* 72: 45-51.
- Packard, C.J., Munro, A., Lorimar, A.R., Gotto, A.M. and Shepherd, J. (1984). Metabolism of apolipoprotein B in large triglyceride-rich very low density lipoproteins of normal and hypertriglyceridemic subjects. *Journal of Clinical Investigation* 75: 2178-2192.
- Paffenbarger, R.S., Kampert, J.B. and Chang, H.G. (1980). Characteristics that predict risk of breast cancer before and after menopause. *American Journal of Epidemiology* 112: 163-168.

- Pariza, M.W. and Simopoulos, A.P. (1987). Calories and energy expenditure in carcinogenesis. *American Journal of Clinical Nutrition* 45(Suppl.): 149.
- Parkin, D.M., Stjernsward, J. and Muir, C.S. (1984). Estimates of worldwide frequency of twelve major cancers. *Bulletin of the World Health Organization* 62: 163-192.
- Passmore, R. and Eastwood, M.A. (1986). *Human Nutrition and Dietetics* (Davidson and Passmore, eds). London: Churchill Livingstone.
- Pastorino, U., Pisani, P., Berrino, F., Andereoli, C., Barbieri, A, Costa, A., Mazzoleni, C., Gramegna, G. and Marubini, E. (1987). Vitamin A and female lung cancer: a case control study on plasma and diet. *Nutrition and Cancer* 10(4): 171-179.
- Paul, A.A. and Southgate, D.A.T. (1978). *McCance and Widdowson's "The Composition of Foods"*. London: HMSO.
- Perisse, J., Sizaret, F. and Francois, P. (1969). The effect of income in the structure of the diet. *FAO Nutrition Newsletter* 7: 1.
- Peto, R., Doll, R., Buckley, J.D. and Sporn, M.B. (1981). Can dietary β -carotene materially reduce human cancer rates? *Nature* 290: 201-208.
- Petrakis, N.K., Ernster, V.L. and King, M.C. (1982). Breast. In: *Cancer Epidemiology and Prevention* (Scholtenfeld, D. and Fraumeni, J.F., eds). Philadelphia: W.B. Saunders, pp 855-870.
- Phillips, R.L. (1975). Role of lifestyle and dietary habits in risk of cancer among Seventh Day Adventists. *Cancer Research* (Suppl.) 35: 3513-3522.
- Phillips, R.L. and Snowden, D.A. (1985). Dietary relationships with fatal colorectal cancer among Seventh Day Adventists. *Journal of the National Cancer Institute* 74: 307-317.
- Phillips, R.L. and Snowden, D.A. (1983). Association of meat and coffee use with cancers of the large bowel, breast and prostate among Seventh Day Adventists: preliminary results. *Cancer Research* (Suppl.) 45: 2403-2408.
- Phillips, R.L., Lemon, R.R., Beeson, W.L. and Kuzuma, J.W. (1978). Coronary heart disease morality among Seventh Day Adventists with differing dietary habits: a preliminary report. *American Journal of Clinical Nutrition* 31: S191-198.
- Pickle, L.W., Greene, M.H., Ziegler, R.G., Toledo, A., Hoover, R., Lynch, H.T. and Fraumeni, J.F. (1984). Colo-rectal cancer in rural Nebraska. *Cancer Research* 44: 363-369.
- Pixley, S., Wilson, D., McPharson, K. and Mann, J. (1985). Effect of vegetarianism on the development of gallstones in women. *British Medical Journal* 291: 11-12.

- Plakké, T., Berkel, T., Beynen, A.C., Hermus, R.J.J. and Katan, M.B. (1983). Relationship between the fatty acid composition of the diet and that of the subcutaneous adipose tissue in individual human subjects. *Human Nutrition : Applied Nutrition* 37A: 365-372.
- Poon-King, T., Henry, M.V. and Rampersan, F. (1968). Prevalence and natural history of diabetes in Trinidad. *Lancet* i: 155-160.
- Potter, J.D. and McMichael, A.J. (1986). Diet and cancer of the colon and rectum: a case control study. *Journal of the National Cancer Institute* 76: 557-569.
- Potter, J.F., Dawkins, D.M., Pandha, H.S. and Beevers, D.G. (1984). Cancer in blacks, whites and Asians in a British hospital. *Journal of the Royal College of Physicians* 18: 231-235.
- Potter, J.F., Terry, P., Dawkins, D.M. and Beevers, D.G. (1983). Breast cancer in blacks, whites and Asians in Birmingham. *Postgraduate Medical Journal* 59: 661-663.
- Preece, M.A., Tomlinson, S., Ribot, C.A., Pietrek, J., Forn, H.T., Davies, D.M., Ford, J.A., Dunnigan, M.G. and O'Riordan, J.L.H. (1975). Studies of vitamin D deficiency in man. *Quarterly Journal of Medicine* 44: 575-589.
- Prentice, R.L., Kakar, F., Hursting, S., Shephard, L., Klin, R. and Kush, L.H. (1988). Aspects of rational for women's health trial. *Journal of the National Cancer Institute* 80: 802-814.
- Prior, I.M. and Evans, J.G. (1970). Current developments in the Pacific. In: *Atherosclerosis II: Proceedings of the Second International Symposium* (Jones, R.J., ed.). New York: Springer-Verlag.
- Reddy, B.S. (1981). Diet and excretion of bile acids. *Cancer Research* 41: 3766.
- Reddy, B.S. (1983). Tumor promotion in colon carcinogenesis. In: *Mechanisms of Tumor Promotion*, Vol.I (Slaga, T.J., ed.). Boca Raton, Florida: CRC Press, Inc.
- Reddy, B.S. and Maeura, Y. (1984). Tumor promotion by dietary fat in azoxymethane-induced colon carcinogenesis in female F344 rats: influence of amount and sources of dietary fat. *Journal of the National Cancer Institute* 72: 745-750.
- Reddy, B.S. and Maruyama, T. (1986). Effect of dietary fish oil on azoxymethane-induced colon carcinogenesis in male F344 rats. *Cancer Research* 46: 3367-3370.
- Reddy, B.S., Cohen, L.A., McCoy, D., Hill, P., Weisburger, J.H. and Wynder, E.L. (1980). Nutrition and its relationship to cancer. *Advances in Cancer Research* 32: 237.

- Reddy, B.S., Tanaka, T. and Simi, B. (1985). Effect of different levels of dietary *trans* fat or corn oil on azoxymethane-induced colon carcinogenesis in F344 rats. *Journal of the National Cancer Institute* 75: 791-789.
- Reddy, B.S., Weisburger, J.H. and Wynder, E.L. (1975). Effects of high risk and low risk diets for colon carcinogenesis on faecal microflora and steroids in man. *Journal of Nutrition* 105: 878.
- Reddy, S. and Sanders, T.A.B. (1987). Plasma phospholipid fatty acid composition in men of Asian and European descent. *Proceedings of the Nutrition Society* 46: 117.
- Reeves, V.B., Matusik, E.J. and Kelsay, J.L. (1984). Variations in plasma fatty acid concentrations during a one year self-selected dietary intake study. *American Journal of Clinical Nutrition* 40: 1345-1351.
- Registrar General (1987). *Mortality Statistics: Cause. Review of Registrar General on Deaths by Cause, Sex and Age in England and Wales 1985*. Series DH2, No.12. London: HMSO.
- Renaud, S., Fodsey, F., Dumont, E., Thevenon, C., Ortchanian, E. and Martin, J.L. (1986). Influence of long-term diet modification on platelet function and composition in Moselle farmers. *American Journal of Clinical Nutrition* 43: 136-150.
- Renwick, A.G. and Drasar, B.S. (1976). Environmental carcinogens and large bowel cancer. *Nature* 263: 234-235.
- Rhoads, G.G., Dahlèn, G., Berg, K., Morton, N.E. and Dannenberg, A.O. (1986). Lp(a) lipoprotein as a risk factor for myocardial infarction. *Journal of the American Medical Association* 256: 2540-2544.
- Roberts, I.F., West, R.J., Ogilvie, D. and Dillon, M.J. (1979). Malnutrition in infants receiving cult diets: a form of child abuse. *British Medical Journal* 1: 296-298.
- Roberts, P.D., James, H., Petrie, A., Morgan, J.O. and Hoffbrand, A.V. (1973). Vitamin B₁₂ status in pregnancy among immigrants to Britain. *British Medical Journal* 3: 67-70.
- Robertson, A., Sprecher, H. and Wilcox, J. (1968). Free fatty acid patterns of human maternal plasma, perfused placenta and umbilical cord plasma. *Nature* 217: 378.
- Robertson, I., Glekin, B.M., Henderson, J.B., McIntosh, W.B., Lakhani, A. and Dunnigan, M.G. (1982). Nutritional deficiencies among ethnic minorities in the U.K. *Proceedings of the Nutrition Society* 42: 243-261.
- Rogers, A.E. and Westel, W.C. (1981). Mammary carcinogenesis in rats fed different amounts and types of fats. *Cancer Research* 41: 3735-3737.

- Rona, R.J. and Chinn, S. (1986). National Study of Health and Growth: social and biological factors associated with height of children from ethnic groups living in England. *Annals of Human Biology* 13(5): 453-471.
- Rona, R.J. and Chinn, S. (1987). National Study of Health and Growth: social and biological factors associated with weight for height and triceps skinfold of children from ethnic groups in England. *Annals of Human Biology* 14(3): 231-248.
- Rose, G., Blackburn, H., Keys, A. *et al.* (1974). Colon cancer and blood cholesterol. *Lancet* i: 181-183.
- Rosen, P.P., Ashikari, R. and Thaler, H. (1977). A comparative study of some pathological features of mammary carcinoma in Tokyo, Japan and New York, U.S.A. *Cancer* 39: 429-434.
- Roshanai, F. (1983). *The Influence of Dietary ω 3 Polyunsaturated Fatty Acids on Vascular Lipids and Platelet Function*. University of London: PhD Thesis.
- Roshanai, F. and Sanders, T.A.B. (1984). Assessment of fatty acid intakes in vegans and omnivores. *Human Nutrition : Applied Nutrition* 38A: 345-354.
- Ross, R., Faggiotto, A., Bowen-Pope, D. and Raines, E. (1984). The role of endothelial injury and platelet and macrophage interactions in atherosclerosis. *Circulation* 70: 77.
- Rybo, E., Bengtsson, C., Hallberg, L. and Oden, A. (1985). Iron status of 38 year old women in Gothenburg, Sweden. Diagnosis of iron deficiency. *Scandinavian Journal of Haematology* 34(Suppl.43).
- Sacks, F.M., Castelli, W.P., Donner, A. and Kass, E.H. (1975). Plasma lipids and lipoproteins in vegetarians and controls. *New England Journal of Medicine* 292: 1148-1151.
- Sacks, F.M., Rossner, B. and Kass, E.H. (1974). Blood pressure in vegetarians. *American Journal of Epidemiology* 100: 390-398.
- Sanders, T.A.B. (1978). The health and nutritional status of vegans. *Plant Foods for Man* 2: 181-193.
- Sanders, T.A.B. (1983). Vegetarianism: dietetic and medical aspects. *Journal of Plant Foods* 5: 3-14.
- Sanders, T.A.B. (1988). *Vegetarian Diets: Briefing Paper*. London: The British Nutrition Foundation.
- Sanders, T.A.B. (1990a). Polyunsaturated fatty acids and coronary heart disease. In: *Lipid and Lipoprotein Disorders* (Betteridge, D.J., ed.). Baillier's Clinical Endocrinology and Metabolism. London: Bailliere Tindall.

- Sanders, T.A.B. (1990b). Dietary fatty acids: effects on lipid metabolism. *Current Opinion in Lipidology* 1: 12-17.
- Sanders, T.A.B. and Naismith, D.J. (1980). The metabolism of α -linolenic acid by the foetal rat. *British Journal of Nutrition* 44: 205-208.
- Sanders, T.A.B. and Naismith, D.J. (1979). A comparison of the influence of breast-feeding and bottle-feeding on the fatty acid composition of erythrocytes. *British Journal of Nutrition* 41: 619-623.
- Sanders, T.A.B. and Purves, R. (1981). An anthropometric and dietary assessment of the nutritional status of vegan pre-school children. *Journal of Human Nutrition* 35: 349-357.
- Sanders, T.A.B. and Younger, K.M. (1981). The effect of dietary supplements of ω 3 polyunsaturated fatty acids on the fatty acid composition of platelets and plasma choline phosphoglycerides. *British Journal of Nutrition* 45: 613-616.
- Sanders, T.A.B., Ellis, F.R. and Dickerson, J.W.T. (1978). Studies of vegans: the fatty acid composition of plasma choline phosphoglycerides, erythrocytes, adipose tissue and breast milk and some indicators of susceptibility to ischaemic heart disease in vegans and controls. *American Journal of Clinical Nutrition* 31: 805-813.
- Sanders, T.A.B., Ellis, F.R. and Dickerson, J.W.T. (1978). Haematological studies on vegans. *British Journal of Nutrition* 40: 9-15.
- Sanders, T.A.B., Hinds, A. and Perreira, C.C. (1989). Influence of n-3 fatty acids on blood lipids in normal subjects. *Journal of Internal Medicine* 225(Suppl.): 99-104.
- Sanders, T.A.B., Mistry, M. and Naismith, D.J. (1984). The influence of a maternal diet rich in linoleic acid on brain and retinal docosahexaenoic acid in the rat. *British Journal of Nutrition* 51: 57-66.
- Sanders, T.A.B., Sullivan, D.R., Reeve, J. and Thompson, G.R. (1985). Triglyceride-lowering effect of marine polyunsaturates in patients with hypertriglyceridemia. *Arteriosclerosis* 5: 459-465.
- Scanu, A.M. (1990). Lipoprotein(a). In: *Lipid and Lipid Disorders*. Baillière's Clinical Endocrinology and Metabolism 4(4): 940-946.
- Scanu, A.M. and Fless, G.M. (1990). Lp(a): heterogeneity and biological relevance. *Journal of Clinical Investigation* 85: 1709-1715.
- Schonland, M. and Bradshaw, E. (1968). Cancer in Natal Africans and Indians 1964-1966. *International Journal of Cancer* 3: 304-316.

- Schultz, T.D. and Leklem, J.E. (1983). Nutrient intake and hormonal status of pre-menopausal vegetarian Seventh Day Adventists and pre-menopausal non-vegetarians. *Nutrition and Cancer* 4(4): 247-259.
- Schultz, T.D., Wilcox, R.B., Spenhler, J.M. and Howie, B.J. (1987). Dietary and hormonal inter-relationships in pre-menopausal women: evidence for a relationship between dietary nutrients and plasma prolactin levels. *American Journal of Clinical Nutrition* 46: 905-911.
- Schwan, A., Rydén, A. and Laurell, G. (1982). Fecal bacterial flora of four Nordic population groups with diverse incidence of large bowel cancer. *Nutrition and Cancer* 4: 74.
- Scott, J. (1989). Lipoprotein(a): thrombogenesis linked to atherogenesis at last? *Nature* 341: 22.
- Seed, M., Hoppichler, F., Reavely, D., McCarthy, S., Thompson, G.R., Boerwinkle, E. and Uterman, G. (1980). Relation of serum lipoprotein (a) concentration and apolipoprotein (a) phenotype to coronary heart disease in patients with familial hypocholesterolaemia. *New England Journal of Medicine* 322(21): 1494-1499.
- Shanmugasundaram, K.R., Suresh, S., Misra, K.P. and Jayakrishnan, T.K. (1983). Plasma lipoprotein cholesterol in Indian in healthy persons and those with coronary heart disease. *Atherosclerosis* 46: 129-135.
- Shaper, A.G. and Jones, F.W. (1959). Serum cholesterol, diet, CHD in Africans and Asians in Uganda. *Lancet* ii: 534-537.
- Shaunak, S., Colston, K., Ang, L., Patel, S.P. and Maxwell, J.D. (1986). Vitamin deficiency in adult British Hindu Asians: a family disorder. *British Medical Journal* 291: 1116-1168.
- Shaw, C. (1988). Components of growth in the ethnic minority population. *Population Trends* 52: 26-30.
- Sheihan, H. and Qucik, A. (1982). *The Rickets Report - why do British Asians get rickets?* London: Haringey Community Health Council.
- Shorter, E.R. (1985). Colorectal cancer in Crohn's colitis and other large intestinal diseases: is there a dysplasia-carcinoma sequence? In: *Carcinoma of the Large Bowel and Its Precursors* (Ingall, J. and Mastromarino, A., eds). New York: Alan Liss.
- Simmons, D., Williams, D.R. and Powell, M. (1989). Prevalence of diabetes in a predominantly Asian community: preliminary findings of Coventry diabetes study. *British Medical Journal* 298: 18-21.
- Simopoulos, A.P. (1987). Obesity and carcinogens: historical perspective. *American Journal of Clinical Nutrition* 45: 271-276.

- Smith, A.H., Pearce, N.E. and Joseph, J.G. (1985). Major colo-rectal cancer. Aetiological hypotheses do not explain mortality trends among Maori and non-Maori New Zealanders. *International Journal of Epidemiology* 14: 79-85.
- Smith, D.J. (1980). *The Facts of Racial Disadvantage: A National Survey 1980*. London: Commission for Racial Equality.
- Smith, W.C.S., Tavendale, R. and Tunstall-Pedoe, H. (1986). Simplified sub-cutaneous fat biopsy for nutritional surveys. *Human Nutrition : Clinical Nutrition* 40C: 323-325.
- Snowden, D.A. (1988). Animal product consumption and mortality because of all causes combined, coronary heart disease, stroke, diabetes, and cancer in Seventh Day Adventists. *American Journal of Clinical Nutrition* 48: 739-748.
- Snowden, D.A. and Phillips, R.L. (1985). Does a vegetarian diet reduce the occurrence of diabetes? *American Journal of Public Health* 75: 507-512.
- Soini, I. (1977). Risk factors of breast cancer in Finland. *International Journal of Epidemiology* 6: 365-373.
- Solomon, E., Voss, R., Hall, V., Bodmer, W.F., Jass, J.R., Jeffries, H.A., Lucibello, F.C., Patel, I. and Rider, S.H. (1987). Chromosome 5-allele loss in human colo-rectal carcinomas. *Nature* 328: 616-619.
- Sorci-Thomas, M., Wilson, M.D., Johnson, F.L., Williams, D.L. and Rudel, L.L. (1989). Studies on the expression of genes encoding apolipoproteins B100 and B48 and the low density lipoprotein receptor in non-human primates. *Journal of Biological Chemistry* 264: 9039-9045.
- Sorokin, M.S. (1973). Myocardial infarction in Fiji. *Medical Journal of Australia* ii: 764-767.
- Spady, D.K. and Dietschy, T.U. (1989). Interaction of aging and dietary fat in the regulation of low density lipoprotein transport in hamster. *Journal of Lipid Research* 30: 559-569.
- Spaet, T.H. (1980). Anaemia is a symptom. Editorial. *Hospital Practice* 15: 17.
- Sprecher, H. (1977). Biosynthetic pathways of polyunsaturated fatty acids. *Advances in Experimental Medical Biology* 83: 35-50.
- Stammers, J.P., Hull, D., Abraham, R. and McFadyen, I.R. (1989). High arachidonic acid levels in cord blood of infants of mothers on vegetarian diets. *British Journal of Nutrition* 61: 89-97.
- Stammers, J.P., Hull, D., Abraham, R. and McFadyen, I.R. (1989). High arachidonic acid levels in cord blood of infants of mothers on vegetarian diets. *British Journal of Nutrition* 61: 89-97.

- Stamp, T.C.B. (1980). Sources of vitamin D nutrition. *Lancet* i: 316.
- Stamp, T.C.B. and Round, J.M. (1974). Seasonal changes in human plasma levels of 25-hydroxyvitamin D. *Nature* 247: 563-565.
- Stampfer, M.J., Willet, W.C., Colditz, G.A., Rosner, B., Hennekens, C. and Speizer, F.E. (1987). Prospective study of diet and cancer in a cohort of women. *Federation Proceedings* 46: 883A.
- Stemmermann, G.N., Nomura, A.M.Y. and Heilbrun, L.K. (1984). Dietary fat and the risk of colorectal cancer. *Cancer Research* 44: 4633-4637.
- Stephen, A.M. and Cummings, J.H. (1980). Mechanism of action of dietary fibre in the human colon. *Nature* 284: 283-284.
- Stephens, W.P., Klimink, P.S., Warrington, S., Taylor, J.L., Berry, J.L. and Mawer, E.B. (1982). Observations of the natural history of vitamin D deficiency among Asian immigrants. *Quarterly Journal of Medicine* 51: 171-188.
- Stewart, J.S., Roberts, P.D. and Hoffbrand, A.V. (1970). Response of dietary vitamin B₁₂ deficiency to physiological oral doses of cyanocobalamin. *Lancet* ii: 542-545.
- Studd, J.W.W., Tuck, S.M., Cardozo, L.D. and Gibb, D.M.F. (1982). Labour in patients of different racial groups. In: *Obstetric Problems of Asian Community in Britain* (McFadyen, I.R.M. and MacVicar, J., eds). London: Royal College of Obstetricians and Gynaecologists.
- Sturdevant, R.A.L., Pearce, M.L. and Dayton, S. (1973). Increased prevalence of cholelithiasis in men ingesting a serum-cholesterol lowering diet. *New England Journal of Medicine* 288(1): 24-27.
- Sturman, S. and Beevers, D.G. (1990). General medical problems. In: *Health Care for Asians* (McAvoy, B.R. and Donaldson, L.J., eds). Oxford General Practice Series, No.18. Oxford: Oxford University Press.
- Tan, S.P., Wenlock, R.W. and Buss, D.H. (1985). *Immigrant Foods, 2nd supplement to McCance and Widdowson's "The Composition of Foods"*. London: HMSO.
- Tannenbaum, A. (1942). The genesis and growth of tumours III. Effects of high fat diets. *Cancer Research* 2: 468-475.
- Tanner, J.M., Whitehouse, R.H. and Tamaishi, I. (1965). Standards from birth to maturity for height, weight, height velocity and weight velocity in British children. *Archives of Diseases in Childhood* 41: 454-457.
- Taussig, M. (1987). Neoplasia. In: *Processes in Pathology and Microbiology*. London: Blackwell Scientific Publications.
- Terry, P.B., Bissenden, J.G. and Condie, R.G. (1985). Ethnic differences in congenital malformations. *Archives of Diseases in Childhood* 60: 866-868.

- The Lancet (1991). Editorial: Lipoprotein(a). *Lancet* 337: 397-398.
- Thom, T.J., Epstein, F.H., Feldman, J.J. and Leaverton, P.E. (1985). Trends in total mortality and mortality from heart disease in 26 countries from 1950 to 1978. *International Journal of Epidemiology* 14: 510-520.
- The Realeat Survey 1984-1990 (1990). *Changing Attitudes to Meat Consumption*. UK: The Realeat Company Ltd.
- Thompson, M.H. (1985). Fecal bile acids in health and disease. In: *Liver, Nutrition and Bile Acids* (Galli, G. and Bosisio, E., eds). New York: Plenum Press.
- Thornton, J.R. (1981). High colonic pH promotes colorectal cancer. *Lancet* i: 1081-1083.
- Thorogood, M., Carter, R., Benfield, L., McPherson, K. and Mann, J.I. (1987). Plasma lipids and lipoprotein cholesterol concentrations in people with different diets in Britain. *British Medical Journal* 295: 351-353.
- Thurnham, D.I., Davies, T.A., Crump, B.J., Situnayake, R.D. and Davis, M. (1986). The use of different lipids to express serum tocopherol: lipid ratios for the measurement of vitamin E status. *Annals of Clinical Biochemistry* 23: 514-520.
- Tinico, J., Babcock, K., Hincoubergs, I., Medwadowski, B. and Milajanich, P. (1978). Linoleic acid deficiency: changes in fatty acid patterns in female and male rats raised on a linoleic acid-deficient diet for two generations. *Lipids* 13(1): 6-17.
- Todd, P.A., Benfield, P. and Goa, K.L. (1990). Guar gum - A review of its pharmacological properties, and use as a dietary adjunct in hypercholesterolaemia. *Drugs* 39(6): 917-928.
- Toniolo, P., Riboli, E., Protta, F., Charrel, M. and Cappa, A.D.M. (1989). Calorie providing nutrients and risk of breast cancer. *Journal of the National Cancer Institute* 81: 278-286.
- Traber, M.G., Ingol, K.U., Burton, G.W. and Kayden, H.J. (1988). Absorption and transport of deuterium-substituted 2R, 4'R, 8'R- α -tocopherol in human lipoproteins. *Lipids* 23: 791-797.
- Tunstall-Pedoe, H., Clayton, D., Morris, J.M., Brigden, W. and McDonald, L. (1975). Coronary heart attack in East London. *Lancet* ii: 833-838.
- Turjiman, N., Goodman, G.T., Jaeger, B. and Nair, P.P. (1984). Diet, nutrition intake, and metabolism in populations at high and low risk for colon cancer. Metabolism of bile acids. *American Journal of Clinical Nutrition* 40: 937-941.
- Turpeinen, O. (1973). Primary prevention of heart disease by diet. In: *Lipid Metabolism and Atherosclerosis* (Cotton, D.W.K., ed.). Amsterdam: Excerpta Medica.

- Tuyns, A.J., Haelterman, M. and Kaaks, R. (1987). Colo-rectal cancer and the intakes of nutrients: oligosaccharides are a risk factor, fats are not. A case control study in Belgium. *Nutrition and Cancer* 10: 181-186.
- Tuyns, A.J., Kaaks, R. and Haelterman, M. (1988). Colo-rectal cancer and the consumption of foods; a case control study in Belgium. *Nutrition and Cancer* 11: 189-204.
- Uauy, R.D., Birch, D.G., Birch, E.E., Tyson, J.E. and Hoffman, D.R. (1990). Effect of dietary omega-3 fatty acids on retinal function of very low birth weight neonates. *Pediatric Research* 28(5): 485-492.
- Underwood, B.A. (1984). Vitamin A in animal and human nutrition. In: *The Retinoids*, Vol.1 (Sporn, M.B., Roberts, A.B. and Goodman, D.S., eds). Orlando: Academic Press.
- United States Department of Agriculture (1979). *Composition of Foods*. Washington DC: USDOA.
- Uriel, J. (1979). Retrodifferentiation and the fetal patterns of gene expression in cancer. *Advances in Cancer Research* 29: 127-174.
- Utterman, G., Kraft, H.G., Menzel, H.J., Hopfweiser, T. and Seitz, C. (1988). Genetics of the quantitative Lp(a) lipoprotein trait. 1. Relationship of Lp(a) glycoprotein phenotypes to Lp(a) concentrations in plasma. *Human Genetics* 78: 41-46.
- Verganic, C. and Bettale, A. (1981). Familial hypo-alpha-lipoproteinemia. *Clinica et Chimica Acta* 114: 45-52.
- Vergroesen, A.J. and De Boer, J. (1971). Quantitative und qualitative Effekte mehrfach ungesättigter und anderer Fettsäuren in der menschlichen Diät. *Wissenschaftliche Veröffentlichungen der Deutschen Gesellschaft für Ernährung* 22: 76-89.
- Verma, N.P.S., Mehta, S., Madhu, S., Mather, H. and Keen, H. (1985). Prevalence of known diabetes in an urban Indian environment: the Darya Gunj Diabetes Survey. *British Medical Journal* 293: 423-442.
- Verreault, R., Bresson, J., Deschenes, L., Nand, F., Meyer, F. and Belanger, L. (1988). Dietary fat in relation to prognostic indicators in breast cancer. *Journal of the National Cancer Institute* 80: 819-825.
- Viegas, O.A.C., Scott, P.H., Cole, T.J., Mansfield, H.J., Wharton, P.A. and Wharton, B.A. (1982b). Dietary protein energy supplementation of Asian mothers at Sorrento Birmingham I: selective during second and third trimester. *British Medical Journal* 285: 589-592.
- Viegas, O.A.C., Scott, P.H., Cole, T.J., Mansfield, H.J., Wharton, P.A. and Wharton, B.A. (1982a). Dietary protein energy supplementation of Asian mothers at

- Sorrento Birmingham II: selective during third trimester only. *British Medical Journal* 285: 589-592.
- Vissek, W.J. (1978). Diet and cell growth by ammonia. *American Journal of Clinical Nutrition* 31: S216-S220.
- Von Staveren, W.J., Deurenberg, P., Katan, M.B., Burema, J., de Groot, L.C.P.G.M. and Hoffmans, M.D.A.F. (1986). Validity of fatty acid composition of subcutaneous fat tissue microbiopsies as an estimate of the long-term average fatty acid composition of the diet to separate individuals. *American Journal of Epidemiology* 123: 455-463.
- Wait, R., Thompson, M.H. and Hill, M.J. (1985). Faecal steroids and colorectal cancer: *allo* bile acids. *British Journal of Cancer* 52: 445.
- Walker, A.R.P. (1963). Extremes of CHD mortality in ethnic groups in Johannesburg, South Africa. *American Heart Journal* 66: 293-295.
- Walker, A.R.P., Walker, B.F. and Walker, A.J. (1986). Faecal pH, dietary fibre intake and proneness to colon cancer in four South African populations. *British Journal of Cancer* 53: 489-495.
- Ward, P.S., Drakeford, J.P., Milton, J. and James, A. (1982). Nutritional rickets in Rastafarian children. *British Medical Journal* 285: 1242-1243.
- Warrington, S. and Storey, D.M. (1988). Comparative studies on Asian and Caucasian children I: Growth. *European Journal of Clinical Nutrition* 42: 69-80.
- Waterhouse, J., Muir, C., Correa, P., Powell, J. and Davis, W. (1976). *Cancer Incidence in Five Continents*, Vol.III. IARC Scientific Publication No.15. Lyon: International Agency for Research on Cancer.
- Waterhouse, J., Muir, C.S., Shanmugaratnam, K. and Powell, J. (1982). *Cancer Incidence in Five Continents*, Vol.4. IARC Scientific Publications No.42. Lyon: International Agency for Research on Cancer.
- Wattenberg, L.W. (1990). Inhibition of carcinogenesis by minor nutrient constituents of the diet. *Proceedings of the Nutrition Society* 49: 173-183.
- Webster, J. and Fox, J. (1989). The changing nature of populations: the British example. In: *Ethnic Factors in Health and Disease*. London: Wright.
- Weiner, B.H., Ockene, I.S., Levine, P.H. *et al.* (1986). Inhibition of atherosclerosis by cod-liver oil in a hyperlipidemic swine model. *New England Journal of Medicine* 315: 841-846.
- Weinstein, I.B., Gattoni-Celli, S., Kirchmeier, P., Hsiao, W., Horowitz, A. and Jeffery, A. (1984). Cellular targets and host genes in multi-stage carcinogenesis. *Federation Proceedings* 43: 2287-2294.

- Weintraub, M.S., Zechner, R., Brown, A., Eisenberg, S. and Breskow, J.L. (1988). Dietary polyunsaturated fats of $\omega 6$ and $\omega 3$ series reduce postprandial lipoprotein levels, chronic and acute effects of fat saturation on postprandial lipoprotein metabolism. *Journal of Clinical Investigation* 82: 1884-1893.
- Welsch, C.W. (1987). Enhancement of mammary tumorigenesis by dietary fat: a review of potential mechanisms. *American Journal of Clinical Nutrition* 45: 192-202.
- Wenlock, R.W. and Buss, D.H. (1977). Nutritional quality of food purchased by Asian families participating in the National Food Survey. *Proceedings of the Nutrition Society* 36: 61A.
- West, R.O. and Hayes, O.B. (1968). Diet and serum cholesterol levels: a comparison between vegetarians and non-vegetarians in a Seventh Day Adventist group. *American Journal of Clinical Nutrition* 21: 853-862.
- West, K.M. and Kalbfleisch, J.M. (1966). Glucose tolerance, nutrition and diabetes in Uruguay, Venezuela, Malaya and East Pakistan. *Diabetes* 15: 9-18.
- Wharton, B. (1982). Food, growth, and the Asian fetus. In: *Obstetric Problems of the Asian Community in Britain* (McFadyen, I.R. and MacVicar, J., eds). London: Royal College of Obstetricians and Gynaecologists.
- Wharton, P.A., Eaton, P.M. and Day, K.C. (1983). Sorrento Asian Food Tables: food tables, recipes and customs of mothers attending Sorrento Maternity Hospital, Birmingham, England. *Human Nutrition : Applied Nutrition* 37A: 378-402.
- Wharton, P.A., Eaton, P.M. and Wharton, B.A. (1984). Sub-ethnic variation in the diets of Moslim, Sikh and Hindu pregnant women at Sorrento Maternity Hospital, Birmingham. *British Journal of Nutrition* 52: 469-476.
- WHO European Collaborative Group (1983). Multifactorial trial in the prevention of coronary heart disease: incidence and mortality results. *European Heart Journal* 4: 141-147.
- WHO Expert Committee (1982). *Prevention of Coronary Heart Disease*. Technical Report Series No.678. Geneva: WHO, pp 1-53.
- Wicha, M.S., Liotta, L.A. and Kidwell, W.R. (1979). Effects of free fatty acids on the growth of normal and neoplastic rat mammary epithelial cells. *Cancer Research* 39: 426-435.
- Widdowson, E.M. (1947). *A Study of Individual Children's Diets*. Medical Research Council Special Report Series No.257. London: HMSO.
- Wilhelmsen, L., Berglund, G., Elmfeldt, D., Tibblin, G., Wedel, H., Pennert, K., Vedin, A., Wilhelmsson, C. and Werkö, L. (1986). The multifactor primary prevention trial in Göteborg, Sweden. *European Heart Journal* 7: 279-288.

- Willet, W.C., Browne, M.C. and Bain, C. (1985). Relative weight and risk of breast cancer among premenopausal women. *American Journal of Epidemiology* 122: 731-740.
- Willet, W.C., Stampfer, M.J. and Colditz, G.A. (1984). Dietary fat and risk of breast cancer. *New England Journal of Medicine* 316: 697-703.
- Willet, W.C., Stampfer, M.J., Colditz, G.A., Rosner, B.A. and Speizer, F.E. (1990). Relation of meat, fat and fibre intake to the risk of colon cancer in a prospective study among women. *New England Journal of Medicine* 323(24): 1664-1672.
- Willet, W. (1989). The search for the causes of breast and colon cancer. *Nature* 338: 389-394.
- Willet, W.C., Stampfer, M.J., Colditz, G.A., Rosner, B.A., Hennekens, C.H. and Speizer, F.E. (1987). Dietary fat and risk of breast cancer. *New England Journal of Medicine* 316: 22-28.
- Williams, C.M. and Dickerson, J.W.T. (1987). Dietary fat, hormones and breast cancer: the cell membrane as a possible site of interaction of these two risk factors. *European Journal of Surgical Oncology* 13: 89-104.
- Williams, C.M. and Dickerson, J.W.T. (1990). Nutrition and cancer - some biochemical mechanisms. *Nutrition Research Reviews* 3: 75-100.
- Williams, C.M., Maunder, K. and Theale, D. (1989). The effect of a low fat diet on luteal phase prolactin and oestradiol concentrations and erythrocyte phospholipids in normal premenopausal women. *British Journal of Nutrition* 61: 651-661.
- Wills, M.R., Day, R.C., Phillips, J.B. and Bateman, E.C. (1972). Phytic acid and nutritional rickets in immigrants. *Lancet* i: 771-773.
- Wing, R.R., Bunker, C.H., Kuller, L.H. and Mathews, K.A. (1989). Insulin, body mass index and cardiovascular risk factors in premenopausal women. *Arteriosclerosis* 9(4): 479-484.
- Wissler, R.W. and Vesselinovitch, D. (1988). Brief overview of the mounting evidence that atherosclerosis is both preventable and reversible. *Journal of Clinical Apheresis* 4: 52-58.
- Wood, D.A., Riemersma, R.A., Butler, S., Thompson, M., Macintyre, C., Elton, R.A. and Oliver, M.F. (1987). Linoleic acid and eicosapentaenoic acid in adipose tissue and platelets and risk of CHD. *Lancet* i: 117-183.
- Wood, P.D., Haskell, W.L., Stern, M.P., Lewis, S. and Perry, C. (1977). Plasma lipoprotein distributions in male and female runners. *Annals of the New York Academy of Science* 301: 748-763.

- Wood, R.A., Butler, S., Riemersma, R., Thomson, M. and Oliver, M.F. (1984). Adipose tissue and platelet fatty acids and coronary heart disease in Scottish men. *Lancet* ii: 117-121.
- Woods, M.N., Gorbach, S.L., Longcope, C., Goldin, B., Dwyer, J. and Morill-LaBrode, A. (1989). Low fat, high-fiber diet and serum estrone sulphate in premenopausal women. *American Journal of Clinical Nutrition* 49: 1179-1183.
- World Health Organization (1990). *Diet, Nutrition and the Prevention of Chronic Diseases*. Technical Report Series No.797. Geneva: WHO.
- World Health Organization (WHO) (1982). *Cancer Incidence in Five Continents* (Waterhouse, J., Muir, C., Shammugarathan, K. and Powell, J., eds). Lyon: WHO.
- World Health Organization (1972). *Nutritional Anaemias*. Technical Report No.503. Geneva: WHO.
- Wynder, E.L., Kajitani, T., Ishikawa, S., Dodo, H. and Takano, A. (1969). Environmental factors of cancer of the colon and rectum. II. Japanese epidemiological data. *Cancer* 23: 1210-1220.
- Ziegler, R.G., Mason, T.J., Stemhagen, R., Hoover, J.B., Schoenberg, G., Gridley, P.W. and Franmeni, J.F. Jr (1986). Carotenoid intake, vegetables and the risk of lung cancer among white men in New Jersey. *American Journal of Epidemiology* 123: 1080-1093.

A P P E N D I C E S

APPENDIX I:

Questionnaire 1 (Screening)

Department of Food and Nutritional Sciences
King's College
University of London

1. Surname _____

First Names _____

2. Address _____

Telephone Home _____

Work _____

3. Date of Birth _____ Age _____

(Please check the above details and correct if necessary)

4. Are you - single ()

married ()

separated ()

divorced ()

widowed ()

5. Do you have any children?

No / Yes

6. What is your ethnic origin?

English	()	Indian	()
Scottish	()	African	()
Welsh	()	Carribean	()
Irish	()	European	() specify:

P.S. The above information will be kept confidential.

7. Do you eat the following foods?

Eggs	_____
Milk	_____
Chicken	_____
Fish	_____
Pork	_____
Lamb	_____
Beef	_____

8. Do you wish to take part in the study?

No / Yes

9. If yes, could you give the telephone number and the convenient time for us to ring and make a further appointment to visit you?

Tel	_____	Time	_____
	_____		_____

QUESTIONNAIRE II:

Questionnaire 2 (Background Information)

DEPARTMENT OF FOOD AND NUTRITIONAL SCIENCES
KING'S COLLEGE (KQC)
University of London
Campden Hill Road
London W8 7AH

Surname _____

First names _____

Title _____

Address _____

Telephone

Home _____

Work _____

Date of interview _____ [_ _ _ _ _]

Subject Number _____ [_ _ _]

1.How old are you? [_ _]

What date were you born? [_ _ _ _ _]

2.Where (which country) were you born? [_ _]

United Kingdom	01	India	04	Other	07
Europe	02	Bangladesh	05		
N.America	03	Africa	06		

3.Where was your father born? [_ _]

4.Where was your mother born? [_ _]

5.In which other countries have you lived before you came to England? [_ _]

6.At what age did you come to England to live? [_ _]

7.What language did you first speak as a child? [_]

English	1	Gujerati	3	Other	
Hindi	2	Bengali	4	European languages	5

8.What other languages do you speak? [_ _] [_ _] [_ _]

9.At what age did you start school? [_ _]

10.At what age did you finish your education? [_ _]

11. Which schools did you attend?

		which country?
Elementary/primary only(5-11yrs)	1 [_]	[_ _]
Secondary (11-18yrs)	2 [_]	[_ _]
College/technical institute(18+yrs)	3 [_]	[_ _]
University/polytechnic(18+yrs)	4 [_]	[_ _]

12. Are your parents alive?

Alive 1
Dead 0

Father

Mother

[_]

[_]

13. If no, what age did they die?

Father

Mother

[_ _]

[_ _]

14. What was the cause of death?

Father

Mother

Accidental	1	Heart disease	4
Cancer	2	Infection	5
Stroke	3	Other	6

[_]

[_]

15. How many siblings do you have?

[_ _]

Brothers

alive [_]

dead [_]

cause of death

[_]

[_]

[_]

[_]

Sisters

alive [_]

dead [_]

cause of death

[_]

[_]

[_]

[_]

16. Are you the (parity no.)

[_ _]

17. Are you married?

Yes 1

No 0

[_]

18.If no,have you ever been married? [_]

19.If yes,how did your previous marriage end? [_]

Widowed 1
Divorced 2
Separated 3

20.Do you have any children? Yes /No [_]

specify:

Age	Sex	Country of birth
	M=1 F=2	
[_ _]	[_]	[_ _]
[_ _]	[_]	[_ _]
[_ _]	[_]	[_ _]
[_ _]	[_]	[_ _]
[_ _]	[_]	[_ _]
[_ _]	[_]	[_ _]

21.How many pregnancies did you have?
Miscarriages [_]
Abortions [_]

22.What is your usual occupation? [_]

Housewife	0	Skilled	4
Domestic	1	Professional	5
Manual	2	Self employed	6
Clerical	3	Business	7

If self employed,how many employees are there? [_]

Is it your/family's business? [_]
Own 1 Family 2

23.What is your husbands usual occupation? [_]

If self-employed,how many employees are there? [_ _]

24.What was your first occupation when you completed your [_]
education?

25.What was your principal occupation before you left for [_]
England?

26.Are you employed at present? [_]
No 0
Yes 1

No.of hrs/week [_]

27.If no,how long have you been unemployed? [_ _]
months

28.Have you been unemployed at any time in the last 12 months?

No 0 [_] No. of months unemployed [_ _]
Yes 1

29.What is/was father's occupation? [_]

30.Is this- your own home 1 [_]
rented house-private landlord 2
council 3

your own flat 4
rented flat- private landlord 5
council 6

boarding with another household. 7

31. How many bedrooms [_]
 living rooms [_]
 bathrooms/toilets [_] do you have?

32. Are the bathrooms/toilets-
 for your own/family's use 1 [_]
 shared with others 2

33. Are the entrances to your bathroom/toilet- [_]
 inside your own front door 1
 outside 2

34. How many people regularly sleep in the house?

Adults(21+) [_]

Children(1-20yrs) [_]

Infants(0-11months) [_]

35. Is your accommodation centrally heated? [_]
 No 0
 Yes 1

36. Do you own a car or van? [_]
 No 0
 Yes 1

37. In the past four weeks have you had any [_]

Fever 1 Diarrhea 4
 Cold 2 Other infections 5
 Sore throat 3

38. Any other sudden illness? [_]
 No 0
 Yes 1 specify

39. Have you been admitted to hospital (as an in-patient) in the past 12 months?

No 0 [_]
Yes 1 specify

40. Were you admitted to hospital before one year ago? [_]

No 0
Yes 1 specify

41. Have you ever been told that your blood pressure was raised?

No 0 [_]
Yes but no treatment 1
treatment discontinued 2
on the treatment at present 3

42. Have you ever been told that you had diabetes?

No 0 [_]
Yes but no treatment 1
controlled by diet 2
treatment - oral drugs 3
treatment - insulin 4

43. Do you suffer from

No 0	Gout	[_]
	Asthma	[_]
	Chronic bronchitis	[_]
Yes 1	Liver disease	[_]
	Jaundice	[_]
	Kidney disease	[_]

44. Do you suffer from pain or discomfort in your chest when you walk uphill or hurry on level ground?

[_]
No 0
Yes 1

45.What do you do if you get it while walking? [_]

carry on 0
slow down/stop 1

46.If you slow down or stop what happens to the pain? [_]

not relieved 0
relieved 1

How soon? [_ _] (min)

47.Have you ever had severe pain across the front of your chest
lasting half an hour or more?

[_]

No 0
Yes 1

48.Has any doctor told you what it is? [_]

No 0 Angina 1 Heart burn 3
 Infarction 2 Heart attack 4

49.When did you last see your doctor for a medical checkup?

Never 0
More than a year ago 1 [_]
Between 12-6 months 2
Less than 6 months ago 3

50. Are you taking any drugs or medicines at present?

Yes [_]

No [_]

If yes,
Name

Frequency

Starting date

51. Do you use any oral contraceptives? [_]

No 0

Yes 1

If yes,

Name or type
Date started

52. Have you ever used any oral contraception in the past?

No 0

[_]

Yes 1

If yes,

Date started
Date stopped

[_ _]
duration in months

53. At what age did your first menstrual period occur?

Below 10 yrs. [_]

13+ to 15 yrs. [_]

10+ to 13 yrs. [_]

Over 15 yrs. [_]

54. Do you have a smear test done regularly? [_]

No 0

Yes 1

How often? [_] yrs

55. Do you smoke? [_]

Yes 1 How many cigarettes per day? [_ _ _]
No 0

56. Did you ever smoke regularly? [_]

Yes 1
No 0

If yes, when did you start smoking?

Date started [_ _]
Date stopped duration in months

57. Do you ever drink alcohol, including drinks you brew or make at home?

No 0 [_]
Yes 1

58. How often do you drink?

Most days 1
3-4 times/week 2
1-2 times/week 3
1-2 times/month 4
1-2 times/6 months 5
1-2 times/year 6
Not at all in the last 12 months. 7

How much have you drunk on any one occasion during the last 12 months?

	Frequency	Quantity units of alcohol
Shandy	[_]	[_]
Beer, Lager, Cider, Stout.	[_]	[_]
Spirits (gin, whisky, rum, brandy, vodka)	[_]	[_]
Sherry or Vermouth (port, dubonnet)	[_]	[_]
Wine (champagne, babycham)	[_]	[_]

APPENDIX III:

Questionnaire 3 (Dietary Information)

Dept of Food and Nutritional Sciences
King's College (KQC)
University of London
Campden Hill Road
London W8 7AH

DIETARY QUESTIONNAIRE

Surname _____

First names _____

Title _____

Address _____

Telephone

Home _____

Work _____

Date of interview _____ [_ _ _ _ _]

Subject number _____ [_ _ _]

1. Who does the shopping for food? [_]

Self	1	Mother-in-law	5
Husband	2	Daughter-in-law	6
Daughter	3	Combination	7
Son	4	Others(specify)	8

2. Who does the cooking regularly? [_]

3. Where do you usually buy your food? [_]

English super market	1
Asian super market	2
Corner shop	3
Combination	4

4. When you cook food with fat and oil, what kind do you usually use?

Ghee	0	Corn oil	4	
Butter	1	Soya or Sunflower oil	5	
Lard or dripping	2	Olive oil	6	
Margarine	3	Blended vegetable oil	7	[_]

specify brand: _____

5. When you buy cooking oil/fat, what weight do you normally buy?

	Quantity	[_ _]
Code units lbs. 1		
Kgs. 2		
Litres 3		[_]

6. How many weeks does that normally last?

No. of weeks [_ _]

7. How often and how much of the following foods do you/did you eat?

Daily 0
Weekly 1
Occasionally 2
Never 3

Foods	Now	Before
<u>Cereals</u>		
Breakfast cereals		
Bread white		
wholemeal		
Chappaties wheat		
millet		
others		
Rice white		
brown		
<u>PULSES</u>		
Moong whole		
dhal		
Chickpea whole		
dhal		
Tuwar whole		
dhal		
Urad whole		
dhal		
Other beans		
Kidney beans		
Blackeye beans		
Others		

Now		Before
MEATS	Lamb	
	Beef	
	Pork	
	Chicken	
	Fish	
	Eggs	
MILK AND MILK PRODUCTS		
Milk	pasteurised whole	
	semi-skimmed	
	skimmed	
Yoghurt	plain	
	flavoured	
Buttermilk		
Cheese		
Cottage cheese		
Cream		
VEGETABLES		
Potatoes		
Sweet potatoes		
Beetroot		
Turnips		
Radish Mooli		
Yam		
Cabbage		

	Now	Before
Cauliflower		
Runner beans		
Butter beans		
Cluster beans		
Peas		
Spinach		
Other green leafy veg		
Okra		
Aubergines		
Bottlegourd		
Bittergourd		
Courgettes		
Pumpkins		
Marrow		
Tomatoes		
Cucumber		
Lettuce		
Celery		
FRUITS		
Oranges		
Apples		
Pears		
Peaches		
Mango		
Grapefruit		
Melons		

	Now	Before
Bananas		
Grapes		
Plums		
Dried fruit		
FATS AND OILS		
Butter		
Margarine		
Hydrogenated veg oil		
Ghee		
Peanut oil		
Safflower oil		
Sunflower oil		
Sesame seed oil		
Corn oil		
Olive oil		
NUTS		
Peanuts		
Cashewnuts		
Pistachio		
Almonds		
Others		
SPICES AND CONDIMENTS		
Ginger		
Garlic		
Chillipowder		
Coriander		

	Now	Before
Cloves		
Cinnamon		
Cardamun		
Cumin seeds		
Mustard		
Pepper		
Asafoetida		
Turmeric		
Tamarind		
SNACK FOODS		
Crisps		
Sweets(Indian)		
Chocolates		
Cakes		
Biscuits		
Bhajias		
Aundhavo		
Dhokra		
Kachori		
Samosas		
Dhai vada		
Vada		
Thepla		
Puri		
Chevda		
Ghantia/sev		

	Now	Before
Chakri		
Farsi puri		
Sev mumbra		
Khajli		
Beverages		
Tea		
Coffee		
Hot chocolate		
Cola		
Lemonade		
Aerated soft drinks		

8. Do you generally eat all your meals at home? [_]

No 0 Yes 1

9. If no, what type of meal do you eat away from home? [_]

Snacks 1
Carried sandwiches/meals(prepared at home) 2
Take-away food 3
Canteen lunch }
 dinner } 4
Combination 5

10.24 HOUR RECALL

I would like to have an outline of what you had to eat and drink yesterday?

Time/meal	Food description	Serving size	Comments

11. Would you say this is your normal daily eating pattern?
No 0 Yes 1 [_]

Yes 1

[]

12.How does your diet differ from the above during weekends?

[illegible]

13. Can you recall a typical weekday diet before you came to live in the United Kingdom?

[illegible]

14. In your opinion how has your diet altered since your living in the United kingdom?

APPENDIX IV:

Nutrient Composition of some Gujarati Snack Foods

APPENDIX 4**Nutrient Composition of some Gujarati Snack Foods per 100 g**

Foods	Energy (kcal)	Fat* (g)	Protein (g)	CHO (g)	Fibre (g)	Starch (g)	Sugar (g)
Aundhavo	216	9.1	5.9	27.7	2.4	26.0	1.7
Cassava chips	253	10.1	0.7	39.8	2.5	39.0	0.8
Chakry	172	9.4	2.1	19.8	0.4	19.3	0.5
Dokra	252	16.2	7.2	19.4	2.6	17.0	2.4
Kichidi	71	2.3	2.6	10.1	0.9	9.8	0.3
Puri	426	25.0	7.0	43.3	4.4	42.0	1.3
Thepla	390	20.5	7.7	43.7	3.4	42.1	1.6

* Fatty acid composition varies with the oil used in preparation.

APPENDIX V:

Statistical Methods

Sample Size and Power Calculations

Sample size calculations were made using the following equation:

$$n = \frac{(Z_{\alpha/2} + Z_{\beta})^2 \sigma^2 (r + 1)}{(d)^2 r}$$

d = the hypothesised difference of the variable of interest

n = the number of subjects per group

σ = the standard deviation of the variable of interest

r = the ratio of controls to cases

Power calculations were made with $\alpha = 0.05$ and $\beta = 0.90$ ($Z_{\alpha/2} + Z_{\beta})^2 = 10.507$.

A sample size of 21 was sufficiently powerful to detect a 10% difference in means if the SD/mean was 0.1, a 20% difference for a SD/mean of 0.2, a 30% difference for a SD/mean of 0.3. Estimates of the SD/mean were taken from previously published data. A sample size of 25 to 30 subjects in each group was aimed for allowing for drop out.

Regression Analyses

Univariate regression analysis was carried out to look for relationships between the biochemical, anthropometric and dietary measurements. As there is a 1:20 chance of finding spurious significant relationships, multivariate analysis was also carried out, which allows for relationships between predictor variables. Where differences between groups were significant in the analyses of variance, group was included as a term in the multivariate analyses. The purpose of the multivariate analyses was: firstly, to test prior hypotheses such as the influence of body build, saturated fat and cholesterol intake on the prediction of plasma cholesterol concentration; secondly it was to help formulate new hypotheses that might be tested. It should be emphasised that the relationships found between biochemical, dietary and anthropometrical variables are not necessarily causally related and should be interpreted with caution.